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CHARACTERS OF CELLS ATTAINING GREAT AGE

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SOME features of the cells of the medulla which continue growth for a century and are active for a much longer period were described in THE AMERICAN NATURALIST in 1926.[1]

It seems highly probable that many other massive cacti in which the shoot is reduced to columnar form would include tracts in which the living cells might attain great age. The extension of the life of the cell over such long periods should afford opportunities for the study of changes in the composition of living matter and in the behavior of the cell in senility, and should also make possible the determination of the principal features which make the continued functionating existence of protoplasm possible.

Attention was turned to the "barrel" cactus or "bisnaga" (*Ferocactus wislizenii*), which is abundant in the region about the Desert Laboratory, in the winter season of 1927. These plants have been used extensively as experimental material in various researches in this laboratory and something is known concerning the behavior of the great mass of cortex under prolonged desiccation. These massive plants accumulate a water-balance so great that they may live without further supplies when kept in a dry room, for periods as long as six years. The

[1] MacDougal, D. T., "Growth and Permeability of Century-old Cells," AMER. NAT. 60, 393–415, 1926.

principal changes of the cortex under such prolonged desiccation will be cited below.

The general structure of one of these plants is shown in Figs. 1 and 2. The reader is also referred to a discussion of the water-storing mechanism by Coville[2], and to the results of the examination of a specimen which was uprooted and kept on a laboratory table for six years by MacDougal, E. R. Long and J. G. Brown. This plant weighed about 38 kg when taken up in November, 1908, and about 26 kg on December 7, 1914. The starvation

FIG. 1. Mature plant of *Ferocactus wislizenii* (Echinocactus) near Desert Laboratory.

and desiccation phenomena present some interesting facts for comparison with the effects of age, as will be discussed in following sections of this paper.

After some preliminary dissections in 1926 the systematic study of a number of individuals was begun in February, 1927. Mature plants are of an ovoid-cylindrical form, 1.5 to 2 m in height, and with a weight of 100 to 225 kilos. The greater part of this mass is made up of the cortex, the central medulla being but a few mm in diameter. Attention was given first to the action with

[2] Coville, F. V., "Desert Plants as a Source of Drinking-water." Smithsonian reports, 1903, 499–505.

especial reference to comparisons with the medulla of *Carnegiea,* previously described, in which individual living cells are active for a century or more.

Anatomical Features of the Medulla

The terminal growing point of the plant is in a depression down into which the external flutings of the cortical tissue and epidermis arch, as shown in Fig. 2[1A]. The central cylinder appears to be attenuated as it nears the apex, so that the medulla does not show until some mm below the growing point. The wall of the woody cylinder

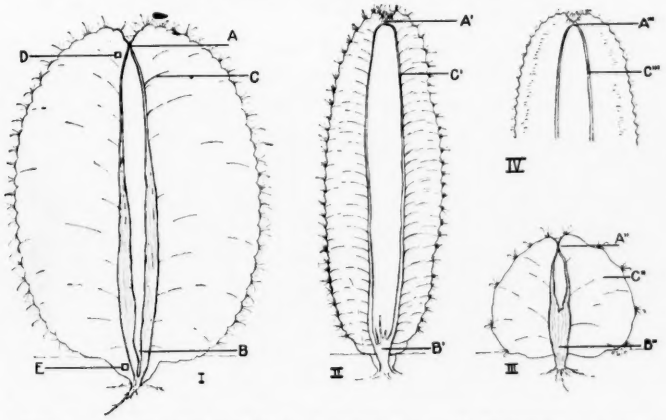


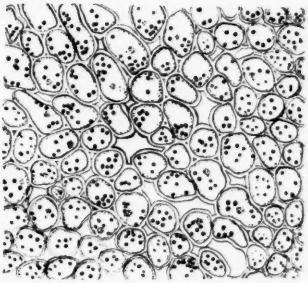
FIG. 2. I, Schematic longitudinal section of small *Ferocactus.* A, apex of medulla, B, base of medulla, C, woody fascicle branching into cortex, D, apex of cortex, E, basal region of cortex. II, Section of *Carnegiea.* A, dome-shaped apex of medulla, B, basal part of medulla, C, Fibrovascular bundles. III, Small *Ferocactus* with regions designated to correspond to those in I. IV, Apical part of trunk of *Carnegiea* with regions designated to correspond to those in II.

enclosing the medulla was 1 to 3 cm in thickness and the rays passing out into the cortex were very thick and wide. Tracts of fibrovascular tissue in great number ramified into the cortical tissue from the central cylinder but not into the medulla, as was the case in *Carnegiea.* Fig. 2[1c]. The medulla was transformed into woody cells at the base, but no tracheids or vessels are seen within the mass of the medulla throughout its length.

The medullary cells in tissue one centimeter from the apex average 90.8 × 57.3 μ in diameter. In cross section these cells are about twelve times as large as cortical cells in the same region, but less than one tenth the size of apical material from medulla of *Carnegiea gigantea*. They are oblong in form, have fairly thick, regular walls and comparatively large intercellular spaces. The distinctive feature of these young medullary cells is the quantity of large starch granules which show up when the tissue is treated with iodine. The nuclei are comparatively large and protoplasmic strands can be seen radiating from them. (Fig. 3).

Practically all the growth of the medullary cells takes place in the upper eighteen centimeters of the stem or within six to eight years after being formed. At that distance from the apex, each diameter of the cells is about twice that of apical cells, averaging 180 × 113.3 μ, so that the general shape of the cells remains the same. Intercellular spaces are smaller and less frequent, and the cell walls thicker, but not regular in thickness. Nuclei are relatively small, very little starch is present, and the cells appear quite empty because of the watery nature of the cytoplasm.

The medullary cells in a cross section 1 cm above the base average 224 in length and 113.3 μ in width. This width averages the same as in the section 18 cm from the apex, but the average length has increased from 180 μ to 224 or about 25 per cent. This elongation of the cells is probably brought about by the compression of the woody cylinder which takes place at this point. Those medullary cells at the base are among the oldest cells in the plant, for they were formed very early in the history of the individual and the new growth has continued to take place in the apical region. They represent an age of 50 to 100 years. Little increase in size has taken place since the first ten years (18 cm) of growth, but these cells have continued to function. The most noticeable change in appearance is found in the cell walls, which show irregular thickening, with very thin places



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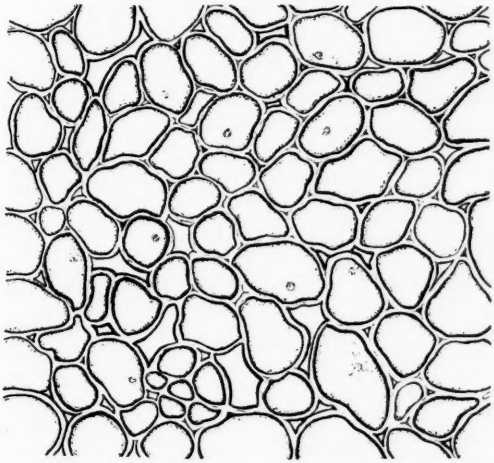

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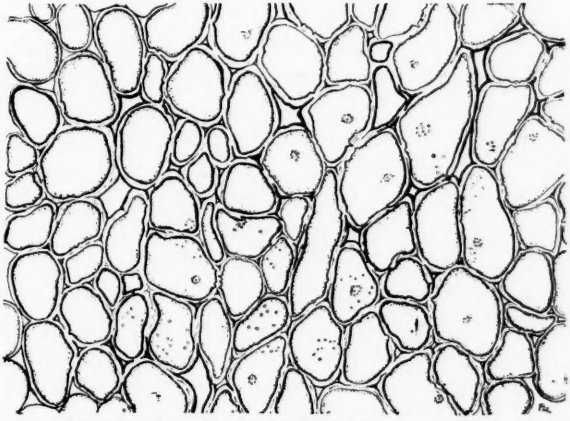


3c

FIG. 3a. Cortical cells of *Ferocactus* less than one year old. Cross section 1 cm. from apex, 26.6 x 16.6 microns.

FIG. 3b. Cortical cells, about eight years old. Cross section 18 cm. from growing point. 180 x 113.3 microns.

FIG. 3c. Basal cells of the cortex, a century old. Cross section 10 cm. above base, 260 x 140 microns

at some points. The nuclei are small, vacuoles very large, and the protoplasm slightly granular in appearance.

The first of several notable differences between the cells of *Carnegiea* and of *Ferocactus* that reach an age of a century or more is that medullary cells of *Carnegiea* continue to enlarge during the greater part of this period, while growth in *Ferocactus* is at an end within a few years.

The retention of the meristematic character of the medullary cells of *Carnegiea* is followed by a persistent transformation of fundamental cells into tracheids and vessels, are previously described. The resulting fascicles ramify densely throughout the medulla of this plant. The long-lived medullary cells of *Ferocactus* lose their meristematic character and no such transformation takes place. Irregular thickening of the wall with perforations takes place in both species with a disappearance of mucilages or wall-building materials from the plasmatic mass.

Experiments with dyes showed that coloring matter was conducted through the central cylinder to the top of the plant within ten hours and that, although the solution passed rapidly out into the cortex, its diffusion into the medulla was extremely slow.

Permeability of Medullary Cells

Plants were taken up from the neighboring mountain slopes late in February and slices of medullary tract were placed in various solutions under the auxograph to test changes in volume. The average of measurements made with material from two plants is given below. The figures given are increases of thickness in terms of the original, or freshly cut cell-masses.

		Apical	Basal
HCl	0.1	Immediate contraction	3 p.ct.
	0.01	4.5	11.5
	0.001	6.8	9.5
Water		7	7.5
KOH	0.001	8.5	12.5
	0.01	9.8	14.5
	0.1	3	16

Another set of tests was made with the medulla of a plant taken from the western slope of the Tucson Mountains on March 20. In addition to measurement of the distention in connection with the H–OH concentration another series was made to test the differential action of the common kations. The results were as follows:

		Apical	Basal
HCl	.05N	Shrinkage	4.5 p.ct.
	.01	2	2.5
	.001	4	0
Water		2	2
KOH	.001	4	7
	.01	7	7
KCl	.001	2.4	7
	.01	2	4.5
NaCl	.001	2.5	5
	.01	2	9
$CaCl_2$	.001	2	12
	.01	1	5.1
KCl	.01	5	
$CaCl_2$	.001		
NaCl	.01		
$CaCl_2$	.001	3.5	

It is notable that both young and old cells of the medulla show a tendency to increased distention as the H or OH concentration increases. This implies that the colloidal layers of the cells are rendered less permeable and that the cells become more efficient osmometers. Not every preparation shows this clearly, but when a multitude of records are made this fact is evident.

It is to be seen that the medullary cells of *Ferocactus* of all ages after maturity (the sections of material tested were all of mature cells) react to H and OH ions like old cells of *Carnegiea*. This may be definitely connected with the loss or decline of the meristematic capacity or at least with age. Young medullary cells of *Carnegiea* show increased permeability as the H or OH concentration increases away from the neutral point.

Results from the use of the artificial cell would suggest that the accumulation of phytosterols in connection with lecithin present from the earlier stages may account for

the lessened permeability and increased distention of aged cells in both plants.

The differential action of the common kations on the medulla of *Ferocactus* also furnished some material for speculation. The amounts of distention of the apical or young cells does not vary greatly in the range of concentration of the salts tested and no significance may be safely attributed to the slight difference in measurements made. It is notable, however, that distention is greater in sodium and potassium solution in the presence of a low concentration of calcium. The lessened permeability and exaggerated distention of basal sections in calcium solution at 0.001 M was very marked.

Some further points of interest are given in the results of tests of relative dry weights, and sap concentration.

Anatomical Features of the Cortex

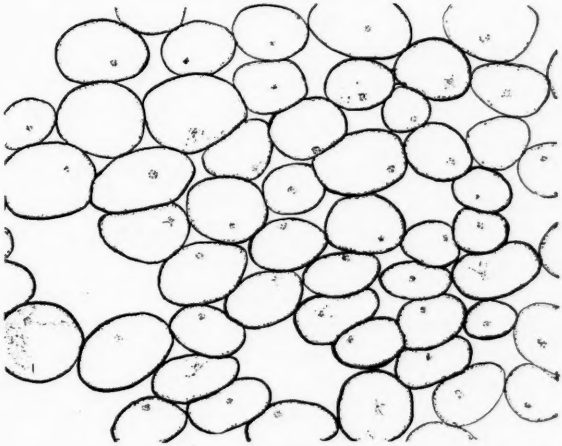
The greatest part of the volume of a plant of *Ferocactus* is made up of the cortex, as may be readily seen by reference to Fig. 4. The external part of this heavy mass is in the form of ribs and furrows well adapted to great variations in volume, but no such differentiation of structure and content of cells is to be found as in *Carnegiea.*

The apical cells of the cortex are spherical to oblong in shape, are thick walled, contain large nuclei and much starch. In a cross section 1 cm from the apex cells averaged 26.6 x 16.6 μ in area.

The cells increase rapidly in size from the apex down the stem and at a point 18 cm from the apex the average diameter was 180 x 113.3 μ, the average cell cross section being about forty times as large as at the apex. At this point the tissue is made up of typical thin-walled parenchyma cells. The intercellular spaces are so numerous and so large that contact points are few, and the cells quite rounded oblong in form. The nuclei are very small, there is practically no starch present, and the cell contents have a very watery appearance.

The cells show a progressive increase in size down the stem from this point and 10 cm above the base, the

4a



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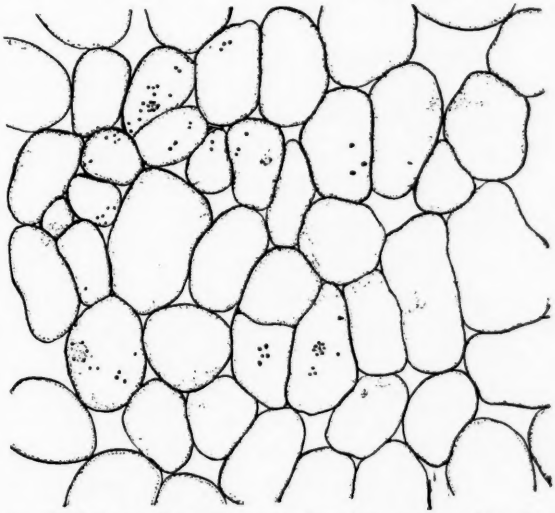

4c

FIG. 4a. Medullary cells of *Ferocactus* less than one year old. Cross section 1 cm. from apex, 90.8 x 57.3 microns.

FIG. 4b. Medullary cells about eight years old. Cross section 18 cm. from growing point, 180 x 113.3 microns.

FIG. 4c. Medullary cells a century old. Cross section 10 cm. above the base, 224 x 113.3 microns.

average diameter being 260 x 140 μ, almost twice what it was at the point 18 cm from the apex. These cells, too, are very thin-walled, but are more closely packed together, there is much less intercellular space and the walls of adjacent cells are more fully in contact. Starch granules are occasionally seen in these cells and sometimes these are grouped around the nuclei. In general these cells present the same empty appearance, except for the nucleus and few protoplasmic strands, that were seen in the section 18 mm from the tip.

Very small crystals are found as cell-inclusions in the various parts of the cortex, but in such small quantity that they are apt to pass unobserved. However, when the expressed sap is allowed to stand, they settle out and can be filtered off. Crystals probably similar in chemical nature to these were found also in *Carnegiea gigantea*, but in such great quantity that they offer a distinct problem in preparing material for histological work. In *Carnegiea* many more and larger crystals were found in the older tissues, which suggests that they probably had some connection with senescence.

It is to be seen from the above that the growth record of cortical cells is distinctly different from that of the medulla. A rapid enlargement takes place in both cortex and medulla during the first ten years of development, the cross section area of cortex cells increasing forty times during this period. The diameter of the medullary cells doubles within the same period. At the end of this initial period of distention the medullary cells are stabilized and show no further definite increases in volume.

The behavior of the cortical cells is markedly different. In the century following the short original period of rapid distention these thin-walled cells show a doubled area in cross sections. A capacity for distention persists throughout this time, but it can not be found that any meristematic action is shown. Meanwhile changes in permeability and in the contents of the cell as determined by dry weight, freezing point of sap, etc., take place as described below.

Sections of cortex of plants in February show changes in thickness under the auxograph as follows: Apical samples were from the youngest part of the cortex and basal from a region near the lower end, the epidermis of which had become hard and brown.

		Apical	Basal
HCl	0.05N	4 Per cent.	Immediate shrinkage
	0.01	10	Slow shrinkage
	0.001	20.7	2
Water		12	2
KOH	0.001	24	1.4
	0.01	17	1.4
	0.05	3	1.2

On March 5 a plant was secured from the slope of the Tucson Mountains 12 miles west of the laboratory and, within two hours, slices from the newer part of the cortex and from the extreme base of the tract were placed under the auxograph. Changes in volume were as follows:

		Apical	Basal
HCl	0.05	Shrinkage	Shrinkage
	0.01	2 Per cent.	5 Per cent.
	0.001	5.6	5
Water		5.6	13
KOH	0.001	16.7	5
	0.01	9	6.6
	0.05	2	3.4
$CaCl_2$	0.01M	13	1

The reactions from the two plants present many features that are different. The apical material of the second sample did not show any maximum distention on the acid side of neutrality, as did the first at HCl 0.001N, but did show a maximum on the alkaline side in KOH 0.001N.

The old cortex from the basal part of the trunk in the first sample was so permeable that distentions were slight and the effects indeterminate. In the second sample distention was very much greater in water than in any solution; the differential effects were not important although there was an apparent maximum in KOH 0.001N.

The figures in the last sample make it evident that a water deficit prevailed in the material and that weak alkali lessens permeability in apical material in some cases,

and that such lessened permeability was also produced by weak acid, Ph 3, in the apical material of the plant previously tested.

Sections from material that had undergone desiccation for four days by lying in the open (the whole remainder of the plant) were hydrated in salt solutions with the following increases:

		Apical	Basal
Water		10 Per cent.	15 Per cent.
KCl	.001	7	23
	.01	13	24
NaCl	.011	9	18
	.01	7	18
$CaCl_2$	.001	7	20
	.01	5.3	16

Another plant, brought in from the south side of the Tucson Mountains, was tested on March 14, 1927. Slices were taken from the older cortex near the base of the central cylinder and from near the apex at a distance well away from the cholorophyllose cells. Changes as follows were measured:

		Apical	Basal
HCl	.0.05	3. Per cent.	5 Per cent.
	0.01	8	10.7
	0.001	12	6.3
Water		6	5.5
KOH	0.001	13	10
	0.01	16	11
	0.01	5	10

The reactions of this plant were much like those of the first; maxima of distention in acid at 0.001N and KOH at 0.01N were shown by apical cortex, and a maximum, that is, distention above that in water was shown by the basal cortex in acid at 0.01N while distention was greater in all alkaline solutions than in water with an apparent maximum at 0.01N.

A notable differentiation between the effects of Ca on the cells from the two regions was evident, as it was also in the plant previously tested.

Differential effects with variations in pH were much more marked in all apical material than in the old cells

of the basal region, and greater differences were produced by the use of identical calcium solutions. These differences suggest that the colloidal material, the permeability of which might be affected by H, OH or Ca ions, is lacking in the old cells.

A second plant brought from the south side of the Tucson Mountains on March 14 was allowed to lie on the ground for three days, then material from the apical and basal cortex was hydrated with salt solutions for comparison with fresh material. The results were as follows:

		Apical	Basal
Water		18 p.ct.	5.4 pct.
KCl	.001M	18	6.5
	.01	16	1.5
NaCl	.001	16	3.4
	.01	14.5	2.7
$CaCl_2$	.001	16	3.6
	.01	11	2

Here, as in the sections previously tested, maximum swelling took place in the apical cortex in KCl .001M and the maximum for basal sections was in the same region of concentration. It was also notable that while the apical cortex showed a distention much less than that in water, in acidified potassium salt the distention in the old cells was much greater than that in water.

While the results of the tests show a wide variation, yet some tentative conclusions may be hazarded. First it is seen that the young cells of the cortex agree with those of the medulla in showing decreased permeability as immersion solutions vary in either direction from the neutral point. This is in contrast with the young medullary cells of *Carnegiea*, which show a greater distention in pure water than in either an acid or alkaline solution. A similar state of affairs was found in some old cells, while in others the results were indeterminate. Further discussion of the points involved is given in the following section.

Variations in Dry Weight, Acidity, Total Carbohydrate and Freezing Point Depression

The medulla from base to apex is rather firm in texture and appears to be quite dry, but dry weight determinations as given below show that it contains a large percentage of water.

	Medulla		Cortex	
	Per cent. water	Dry Per cent. weight	Per cent. water	Dry Per cent. weight
Apex	89.8	10.2	91.4	8.6
Base	87.4	12.6	93.7	6.3

In *Carnegiea* the medullary cells at the apex contained 5 per cent. more water than those from the base and in this species the difference was accounted for not only by the enlarged cells, but also was due to the notable proportion of vascular elements. *Ferocactus* is apparently uniform throughout in type of cells and the difference in dry weight may be accounted for by the thickening of the walls and increase in cell size.

Material from the different parts of the plant was cut into strips and then run through a meat grinder before expressing. All the material could easily be expressed with the hand press, which was quite different from material of *Carnegiea,* which, for the most part, had to be squeezed and rubbed through cloth bags, due to its mucilaginous nature. No enzyme action, as evidenced by color change took place in this material, but medullary material from *Carnegiea* changes rapidly when exposed to the air.

Titrations of the expressed sap with KOH 0.1N showed it to be only very slightly acid, requiring .08 ml KOH to neutralize 1 ml sap in all regions except outer cortex base, which required .128 ml per ml sap. Here we have an example of young apical cells in the medulla, and basal cells, perhaps a century old, in all of which the total acidity differs but little. In *Carnegiea* the acidity of the

young apical cells was greater than in the old cells at the base.

Microscopical examination of the cells in the different regions, using iodine as a stain indicated the presence of a large amount of starch in both the medulla and cortex at the apex. Chemical analyses of the expressed sap, using Fehling's solution and final titration with thiosulphate,[3] gives the following results:

	Mgs. total carbohydrate as dextrose per cc. juice	
	Juice as expressed	Hydrolyzed juice
Medulla		
Apex	.1533	.3710
Base	.0000	.0217
Outer cortex		
Apex	.2080	.3236
Base	.1376	.2498
Inner cortex		
Apex	.1200	.1768
Base	.0840	.1400

This table shows that the young cells at the apex of the medulla are high in carbohydrates, but the greater part of these are of the hydrolyzable type, probably largely made up of starches, as the microscopical examination indicates. In contrast to this no carbohydrates were found in the juice as extracted from basal cells and only 5 per cent. as much carbohydrates resulted from the inversion as was found in the apical region. The inner cortex at the apex contained less carbohydrate than the medulla in the juice as expressed, but the outer cortex contained 25 per cent. more than the medulla, as might be expected, since that is nearest the active photosynthetic region. At the base, the sap from the outer cortex is high in total carbohydrates, but lower than the sap from the apical regions and much higher than the juice from the medulla.

Freezing point depression determinations were made with the Beckman thermometer according to the method

[3] Spoehr, H. A. Carbohydrate Economy of the Cacti. Carnegie Inst. of Wash., 287: 33–36.

of Gortner (*Plant World* 17: 49–53, 1914) and the table found in *Amer. Jour. Bot.* (1914, 78) was used for the conversion of degrees of depression into osmotic pressures in atmospheres. The results were as follows:

Region	Tissue	Depression of Freezing Point in Degrees Cent.	Calculated as Osmotic Pressure in Atmospheres
Apical	Outer cortex	0.460	5.543
	Inner cortex	0.370	4.459
	Medulla	0.520	6.266
Basal	Outer cortex	0.400	4.821
	Inner cortex	0.380	4.580
	Medulla	0.400	4.821

The plant used was cut March 6, 1927, at the close of a season of good rains and about the time when the days were beginning to be much warmer. The apical and basal material used was that from the extreme ends, in so far as possible, although the medulla was so small in diameter (less than a cm at the ends and 4 cm at its greatest diameter) that pieces 15 to 20 cm in length were needed to secure the required amount of sap.

The variation in the depression of the freezing point in the basal material from the different tissues was very slight. There is a difference of 1.80 atmospheres between the medulla and the inner cortical material at the apex, although these tissues at this point are separated from each other only by a very delicate thin layer of the woody skeletal ring. The difference of 0.723 atmospheres between material from the outer and inner cortex is probably due to the fact that outer cortex is the active photosynthetic layer. The medulla in the apical region has a depression of 1.445 atmospheres more than the material from the base, a difference which corresponds to the carbohydrate storage in those parts.

Comparing the amount of depression in the various regions of the *Carnegiea gigantea* with the depressions of the same regions in *Ferocactus wislizenii* we find a much wider range of variation in the former species. In

sap from the medulla of *Carnegiea* of which determinations were made March 8, 1926,[4] the osmotic pressure was 1.16 atmospheres higher in the apical region than in the basal portion. Determinations made May 18 and December 1, 1926, showed the reverse conditions or a higher concentration at the base of the medulla. Sap extracted from the cortex, both inner and outer regions, showed a higher concentration at the apex in *Carnegiea* while in *Ferocactus* there is a higher concentration in the outer cortex at the apex and practically the same pressure for base and apex of the inner cortical region.

Discussion

The facts presented on the foregoing pages include the general anatomical features of the medullary and cortical tracts of *Ferocactus,* a massive cactus with an ovoid-cylindrical stem. Some of the cells in these tracts are seen to persist in a functionating condition for periods of a century or more, after the manner of the elements of a larger, or tree cactus, described in a previous paper. The changes observed in these long-lived cells in the course of a century have been determined by noting the progressive differences in a series of cells of different ages in young and old plants. There also are available the results of a previous study by MacDougal, E. R. Long and J. G. Brown[5] of the changes which had taken place in a *Ferocactus* which had been uprooted and kept on a bare laboratory table in diffused light and without a water supply for six years.

The chief interest in such study concerns the matter of senescence. Never before have such long-lived cells been considered with reference to changes with age. All of the observations have been directed to the general anatomical features of the cells, course and amount of

[4] MacDougal, D. T. Growth and Permeability of Century Old Cells, AMER. NAT. 60, 402, 1926.

[5] MacDougal, D. T., E. R. Long, and J. G. Brown. End Results of Desiccation and Respiration in Succulent Plants. Physiol. Researches. 1. No. 6, 1915.

growth, permeability, hydration capacity, changes in the principal plasmatic constituents, and cell-sap, acidity and dry weight. Minute structure of walls, plasmatic layers and nuclei have not been taken up, as a study of this kind is being made in another laboratory by arrangement.

While the cells are active over a period of a century or more, environmental conditions are favorable to growth over a period not to exceed 200 days of each year. Respiration goes on at a moderate rate during the entire year.

The medulla of *Carnegiea* has been shown to retain the power of growth and some meristematic capacity, while the cortical cells of *Ferocactus* retain the power of growth but not of divison or transformation for periods estimated as well over a century. Graphs expressing the course of growth in the two cases would be quite dissimilar. That of *Carnegiea* shows the rapid distention of young cells for a year or two, then a slow increase for about a half century when the rate rises the life of about a half century when the rate rises until the end of the life of the plant during another half century or more. The rate of distention of the cortical cells of *Ferocactus* is very rapid during a period well within the first ten years, during which time the area of the cross section increases about forty times. The increase during the remaining century or more is scarcely more than 100 per cent. as measured by the area of cells in cross section at the end of the phase of rapid growth. At this time, however, the cells have an area in cross section 80 times as great as that which they show during the first year or two.

The medullary cells attain full size in masses within 18 cm of the growing point or within ten years of the time of their formation. Practically no change in volume is seen in the remaining century of their existence. Some compression takes place by which deformation but no increase in volume can be asserted. The first medullary cells formed in young plants are transformed into wood but this capacity does not appear to persist, so there is no progressive change in the medulla as in *Carnegiea*.

There is a progressive diminution of the plasmatic colloids in all three cases. Pentosans or mucilaginous material seems to disappear from the protoplasm in the medullas of the two plants coincidentally with progressive thickening of cell-walls.

Thickening of cells does not take place in the cortex of *Ferocactus.* On the contrary, the dry weight of cortical cells decreased with age as carbohydrates and other material from the protoplasm was consumed without being converted into wall material. In consequence the osmotic value of the sap of basal cortical cells is less than that of the apical region.

Wasting of carbohydrates would of course be expected. This was emphasized in the earlier studies of the effects of starvation and desiccation in which the proportionate dry weight of cortical material which had been deprived of water for six years was not materially changed, the wasting of the carbohydrates, including wall material, keeping pace with loss of water.

The starvation experiments showed but little decrease in the protein content of the tissue in six years, although the mass of protoplasm was noticeably diminished. It was likewise seen that the proportion of lipoids to the dry weight of the tissues remained unchanged. It seems highly probable that in the senescence of the medullas of *Carnegiae* and of *Ferocactus* and in the cortex of the latter a similar program is followed. The relative swelling and the reactions of young and old cells would indicate the diminution of the pentosan content of the cells of *Carnegiea,* a matter which may be verified by touch and by analysis. This feature is not so obvious in *Ferocactus,* since the initial proportion of pentosans or mucilages is much less, as evidenced by the reactions of living cell-masses to H and OH ions. Thickened cell-walls may almost invariably be taken to predicate the transformation of mucilages from the plasma.

Information concerning the lipoids is not so clear. The proportion is seen not to diminish. What changes may

take place in the lecithin-like substances can not be profitably discussed beyond the suggestion that reactions with calcium may bring these substances into different combinations with advancing age, while the striking changes in permeability in dilute potassium solutions indicate combinations with this element which change permeability in a very pronounced manner.

Permeability increases with age. The wasting of the jellies of the plasma and in the wall may bring this about, as is illustrated in some of the experiments described on the preceding pages. Greater distentions by hydration were shown only in masses of basal or old cells which had been partially desiccated than in young ones. Increased permeability may rest upon not only the loss of mucilages but also in the enlargement of perforations in walls which would be imperfectly filled by the depleted plasmatic strands.

The wasting of protoplasm may be taken as a general accompaniment of senescence of the plant cell which may or may not result in thickening in cell-walls. In this decrease of the volume of the protoplast the pentosans diminish most rapidly. In starved plants the lipoids and proteids have been known to retain their original proportions to the dry weight, but when other features are considered this may also mean a lessened amount of materials of both classes. It seems eminently reasonable that in plants such as the cacti, in which respiration is essentially of a carbohydrate character, such substances should be wasted in senescence. The amount of insoluble matter in the form of crystals or other insoluble inclusions generally increases with age. The proportion of total soluble matter decreases with age, and also the total titratable acidity. The picture of the century-old cell is that of a machine in which some of the parts have been badly worn, wastage not being replaced by new material and the composition of the basic structure weakened by the disproportionate oxidation of one of its components. How far the lecithins have become modified by combination with potassium can not be stated.

Summary

The principal points of interest described in the foregoing paper are as follows:

(1) Cells of the medulla and of the cortex of the massive cactus *Ferocactus wislizenii* (*Echinocactus wislizenii*) have been found to live for a period of a century or more, something after the manner of the medullary cells of *Carnegiea.*

(2) Medullary cells reach full size within the first decade and undergo some deformation but no measurable change in volume in the following century.

(3) Cortical cells make an increase by which the area of their cross section would be magnified forty times during the first decade. The average areas of cross sections of cells is doubled in the following century consequent upon a slow growth maintained during this period.

(4) Neither medulla nor cortex retain meristematic capacity during the extended life-period as in the case of *Carnegiea.*

(5) Increase of the H or OH concentration in hydrating solutions shows a maximum distention and a minimum of permeability in both young and old cells of the medulla. The maximum on the acid side is in solutions in pH 2–3 and on the alkaline side at about P*h* 10–11. Medullary cells showed high distention following low permeability in calcium solution at 0.001M, with a lesser effect in a similar potassium solution.

(6) The maximum distention of cortical cells with varying H, OH concentration was definitely nearer the neutral point than in the medulla. The critical regions were found in HCl at 0.001N and KOH at 0.01–0.001N. These regions or zones were not well defined in old cells.

(7) Definite maxima of distention of both young and old cortical cells, indicative of minimum permeability were found in immersion solutions of potassium at 0.01–0.001M, while the effects in calcium were not so well defined. This effect is to be compared with that of calcium on medullary cells. Such results may find an explanation in the differing constituents of the two tissues.

(8) Relative dry weight increases with age in medullary cells. Relative dry weight decreases in cortical cells with age.

(9) Carbohydrates decrease with age in both medulla and cortex.

(10) The osmotic value of the cell-sap diminishes with age in both medulla and cortex.

(11) Permeability of cell-masses of both cortex and medulla increases with age, coincidentally with wasting of the protoplast and accentuation of the perforations of walls.

(12) Increase in crystals and insoluble inclusions is seen in both medulla and cortex with age.

(13) The results described are supported by previous tests indicating that the pentosan component of protoplasm is wasted in senescence and that lipoids and proteins undergo lesser change with age.

(14) The respiration of plants being essentially of a carbohydrate character, the total titratable acidity of the cells shows a diminution with age.

QUANTITATIVE METHODS IN GENETIC RESEARCH[1]

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IN the old days, and still to-day, many people enter biology as a refuge against the rigors of physical science, and as a rendezvous of romance. Living things are plastic, moving, changeable, full of delightful surprises and pretty paradoxes, unexpectedly responsive, perhaps unknowably sentient. Even their chemistry has long been indefinite and empirical. They have seemed, in practice at least, indeterminate. And so it is commonly felt that the picture of them painted by the modern geneticist is just "too good to be true"—or, shall we say, "too true to be good"—that living things just can not, and, anyway, do not, work with such precision and automaticity as he claims, and that even if they should, it would not be in good taste to care to have anything to do with them. And so the geneticist becomes, in some quarters, rather an outcast; he is given a separate genetics section, and is supposed to be a narrow-sighted specialist, unfit to teach general biology. Let him fence in his neat domains, it is said, and not encroach upon the traditional happy hunting grounds of more liberal-minded obscurantists. Now, what has brought genetics to such an enviable, if not covertly envied, position, so comparable with that of physical science?

The most productive efforts in genetics, as in physical science, have involved careful quantitative work in which three features have usually been combined: precise measured observation, controlled manipulation and creative

[1] Paper read in symposium on "Quantitative Biology," at joint meeting of the American Society of Naturalists and the American Society of Zoologists, Philadelphia, December, 1926.

calculation (using this word in its broadest sense). Weakness in any of the legs of this tripod has generally resulted, temporarily, in a faulty superstructure, but strength in all three has each time led to sound advances. Due to the defective heritage of biology in general, the feature commonly the weakest has been that of calculation, and to this weakness most of the aberrations of the science have been due; by contrast, in the advances, this element has been the most conspicuous.

Thus, in the establishment of Mendel's two laws, not only were accurate counts and controlled crosses necessary, but also an analytical and mathematical mode of procedure, both in the planning of the operations and in the interpretation of the results; otherwise the processes of mathematical permutation at work here, which, as Harris once pointed out, really underlie the ratios, would not have been discovered. As it was, laws were disclosed somewhat similar to the chemical laws of definite proportions and of multiple proportions, and which, like them, led to the postulation of unit-particles.

Later, in the identification of these units with visible cell structures, a similar mental attitude was necessary. Chromosomes, sex-chromosomes and reduction had long been known, but strong evidence for their constituting the hereditary material was not forthcoming before the necessary results of their behavior were figured out. As Sutton showed, the manoeuvers of the chromosomes would lead to ratios more than curiously like those of "factor" recombinations. And the brilliant inference of McClung, later so ably confirmed by Stevens and by Wilson, capped the climax by showing that, in the case of the sex chromosomes, a logical following out of consequences and a joining together of the individually known steps of chromosome permutation not only led to the same sex ratios as produced in nature, but proved a complete identity of distribution of the chromosomes in question and genetic sex determiners, which behaved in a manner essentially Mendelian. Here again, then, it was calcula-

tion, applied to the results of observation and experiment, that won the battle—not intricate calculation, yet of a type so far from the beaten path of biological thought that it must have appeared to many like driving mathematical procedure too far in its application to living things.

The next great achievement along this line lay in the mapping of these chromosomes. Previous to this, Bateson and Punnett, by the routine application of Mendel's classical quantitative methods, had found cases in which his second law—random assortment—did not hold. Other such cases, of "incomplete linkage," as we now term them, were soon found, and finally Morgan found such cases in Drosophila, in which, by application of the processes of reasoning that McClung had used for sex, the genes concerned could be shown to be associated with a given—with *one* given—chromosome (again the X chromosome), and he pointed out that the cytologist Jansenns' previously constructed "chiasma-type hypothesis" provided a mechanism that would explain the different linkages in such a case. Sturtevant, thereupon adopting the essentials of Jansenns' proposal as a working hypothesis, had the revolutionary idea that by refined quantitative determinations of the amounts of linkage he might be able actually to plot the relative *positions* of the genes within the chromosome, and further, that the ability of his map to work consistently, in predicting previously untried linkages, would provide a test of the assumptions on which the mapping was based. The sensational success of Sturtevant's work is well known. Especially for short distances the linkage relations agreed closely with the spatial relations of points in a line, and, as he has recently shown in his study of bar eye, this finding holds even for directly adjacent genes. On the other hand, apparent discrepancies such as were noted in the case of larger distances were to be expected if double crossing over could occur. All this was a triumph of "the quantitative method."

The present author, in an attempt at a more detailed analysis of the numerical relations in linkage, suggested per cent. of crossing over as a *measure* of chromosome distance, mathematically more suitable than the ratio of non-crossovers to crossovers that had been in use since Bateson's work, and showed that the observed deviations of the large linkages from the map distances were better expressed in terms of a coefficient, based on this mode of measurement, called "coincidence" (sometimes conversely referred to as "interference"). This coincidence was shown to vary in relation to the map distance in a way that could be physically explained only on the so-called "mechanical theory of crossing over," involving a physical interchange of sections of a linear solid body having certain properties of rigidity, a result that I have designated as "the law of linear linkage." Weinstein's important work on coincidence gave further evidence of this. Failure on the part of some geneticists to understand the quantitative relations here involved led to a number of ill-considered attacks on the theory of crossing over, under the cloak of a more liberal conception of physiology, of immunity, of geometry or of cell division, as the case might be, but these substitutes lasted only where and so long as they were not called upon to fit the actual numerical relationships in linkage and coincidence.

In succeeding years, the accumulation of more such quantitative data enabled these findings to become generalized for more chromosomes, and, starting with Altenburg's work on linear linkage in Primula, for widely scattered species. The collective results further caused other principles gradually to emerge, which were in harmony with the foregoing—such are the principles of the correspondence between the numbers and sizes of the linkage groups, and the lengths of their maps, on the one hand, and the numbers and sizes of the chromosomes on the other, even as in physical science the periodic system as arrived at through detailed chemical data corresponds with the one based on physics. This work of painstaking

accumulation of data, in which Bridges has stood pre-eminent, has been most eventful in the discovery of various aberrant cases—at first nearly all were found by Bridges—and these chromosome misplacements, deficiencies, translocations, etc., turned out indeed to be the exceptions that proved our rules, and at the same time illustrated further capabilities of the hereditary material. The theoretical work, or "calculation," necessary in diagnosing and then in following up these cases (shared in, for Drosophila, by Sturtevant, Bridges and the author in cooperation) may not employ the classical symbols and steps of general mathematics, but its processes are of a comparable type, and the data with which it works must certainly be quantitative. The work of Federley on butterflies, and that of Blakeslee, Belling, Baur, Emerson and others on plant material, comes under the same category. Justice can not be done in this brief account (with its inescapable Drosophila emphasis) to the great volume of important genetic work of a quantitative character done by these investigators and by Castle, Wright, Goldschmidt, Bateson and Punnett, Mohr, and many others, in strengthening and extending these basic principles.

Now while this material foundation of gene behavior was being worked out, other quantitative work was being done in the refinement and universalization of the concept of the genes. Thus, Johannsen's model quantitative studies on variation within and among different homozygous groups had proved, for his cases, the great stability of the gene in the absence of crossing; the inference was that most hereditary differences within families that result from crossing are produced by Mendelian segregation. There remained, however, numerous objectors, who found in some crosses, like that of the Negro with the white man, for instance, results that they regarded as contradictory to the strict idea of stable, unmixing genes. It is now a familiar story how, by the application of a quantitative analysis more refined than that known to Mendel, it was proved by East, Shull, Nilsson-Ehle, Tine

Tammes and others that the gene differences affecting a given character may be numerous and cumulative, and so necessitate a more complicated, statistical form of reckoning, with the production of a continuous curve of variation. Similar cases were, independently of the above, worked out by Altenburg and the present writer, who were the proponents of the "multiple factor" conception in the Drosophila investigations, but here there were the additional complicating features, simultaneously present, of lethal effects and differential fertility, which required a use of the newly forged weapons of linkage. The repercussion of these findings upon the seemingly unconformable Oenothera problems can not, for lack of time, be dwelt upon here. Neither can the clearing up of the outstanding alleged cases of "impure segregation," which claimed to rest upon a quantitative basis, but in which the element of calculation had in fact been defective. The associated problem of heterosis—so long the camping ground of the mystics—likewise found its explanation in the multiple gene-differences involving vigor, thanks to the extensive analytical crosses of Jones, East and others; and even in the cross of the white man by the Negro, it was shown by Davenport, despite the difficulties of the study, that the numerical relations of the color classes, which had been separated from each other by objective measures, exhibited the peculiar characteristics to be anticipated for a case of cumulative, semi-dominant, but definite segregating genes. In this and other work Davenport showed the transcendently important phenomena of human inheritance to have the same properties as inheritance in other forms, so far as the refractory material could be expected to show anything at all.

All the work so far reviewed is now an old story; what are we to expect of quantitative methods in genetics in the present and immediate future? The arrangement and mode of distribution of genes in normal, and in many types of abnormal cases, is now largely known, and the mere repetition of cases, though often of great practical

utility, becomes dreary from the theoretical standpoint. There is always the chance of hitting something of value in new abnormal cases of chromosome derangement, but it is becoming more and more a gambler's chance.

It is suggested on many sides that the next organized line of attack must be "phaenogenetics"—a study of the mode of operation of the genes in affecting development and physiology, to be carried on by means of quantitative measurements of the effects—chemical, physiological and morphological—of given gene differences, at different stages of development, and under varied conditions. Such studies, like those of Onslow, of Krafka and others of Zeleny's students, of Haecker, and recently of Plunkett, can not fail to be enlightening eventually from the standpoint of physiology and morphogenesis, but it is dubious whether they can soon succeed in proving anything about the nature of the immediate reactions participated in by the genes directly, still less whether they can prove anything about the nature of the genes themselves, for, as multiple factor theory as well as developmental mechanics and physiology shows, the chains of reactions initially set in motion by the genes may have a myriad steps and interconnections. When we find, for instance, that a certain gene difference results in the presence or absence of a particular enzyme, we have not proved that the gene directly produced that enzyme; it may merely have caused, through a series of intermediate processes, the production of an acid that activated or destroyed that enzyme, the acid having in turn been produced by another enzyme, and that activated by a coenzyme, and that produced by a protein—when the latter was ionized by the gene! Who can tell, in this house that Jack built? There are, to be sure, plausible reasons for thinking of the gene as enzymatic in nature, but the matter can hardly be proved by the reaction-curves, etc., found in "phaenogenetic" studies, and biochemistry may have to forge new weapons before work of this school can trace the way back to the real physiology and chemistry

of the gene itself. But, however that may be, such analytical studies must become increasingly important for general biology, which can not indefinitely ignore its thousands of gene-inhabitants, the carpenters of its house. Thus geneticists will be required more and more to be armed with all the quantitative equipment of physics and chemistry, with their attached mathematics. Genetics is beginning to knock at the door of general physiology and developmental mechanics to-day, and, if they do not open it now, they will in time come knocking on the other side, and instead of genetics finding itself an adjunct of physiology, physiology will find itself an outgrowth of genetics.

Meanwhile, before such detailed causal analysis can proceed very far, a quantitative study of the final character-effects of hereditary differences, and a comparison of them with each other and with environic effects, remains of considerable importance. Due chiefly to Wright's remarkable development of the theory of path coefficients, it is now possible to solve the nature-nurture problem in any given case in which appropriate breedings can be resorted to, and to state the answer in numerical terms that make the fruitless discussions of the past appear ridiculous. Even in the case of human traits—chiefly by the aid of studies on identical twins, which here replace the inbred lines—the way is open for a quantitative attack on these problems.

Turning, now, from such questions of phaenogenetics, we find another promising new line of quantitative work in the synthetic treatment of the known principles of Mendelian inheritance, in order to ascertain the effect of various systems of breeding and selection on individuals and groups of individuals after the passage of some specified large number of generations. This work is unfortunately too little known as yet to general biologists, for here the most advanced types of mathematics yet used in genetic work are required. The studies of the effect of different systems of mating on the amount of homozy-

gosity, so ably started by Jennings and by Fish, and brought into a magnificent generalization by Wright, are the best known examples of such treatment. The calculation, by Fisher, of the manner in which the mutant gene number fluctuates in random breeding, which I think deserves to be called "Fisher's law," is equally significant in showing how evolutionary changes involving "latent" or indifferent mutant genes must proceed. The effect of selection, acting in various ways upon genes of various types of manifestation, have been studied by both Fisher, Haldane and Wright, and the results presented in the form of illuminating curves. Such reckonings can be checked by observation and experiment, where necessary, for—as work of Harris has shown—the results even of natural selection may be studied quantitatively. A great deal remains yet to be done in such lines, but so we see the implacable machinery of quantitative genetics gradually mowing its way through the forests of evolution and ecology, into the rocks of paleontology, and over the graveyard of comparative anatomy.

Is it not obvious that for genetics, more and more, mathematics is becoming necessary, and that even the much calumnied branch of it called biometry is coming into its own? The direct effect of the latter on genetics was at first small, but the quantitative spirit fostered by Galton nevertheless spread across to genetics and helped to give it the attitude of an exact science. Partly, biometry remained self-isolated, because of the hemiopia of some biometricians, but now we see that wherever that which we call "chance" is involved—and that is pretty generally in genetics—some form of biometry is requisite. Entire theories of genetic variation, and a whole theory of sex determination have had to be consigned to the scrap heap because of lack of understanding of what constitutes a significant difference, or of how to determine dependent variation.

Of course, even if one is armed with all the highest refinements of mathematical formulation, the mere routine

application of the formulae to case after case will not necessarily continue to bring in valuable results in an already much worked field. And, on the other hand, it is sometimes true, where we are not concerned with the resultant of many variable elements of diverse tendencies, that a fundamentally important result may be attained in an experiment involving no counts, measurements or strictly mathematical computations at all. Genetics has some examples of this. There is no sure or constant formula for successful research, and creative analysis, plastic and adaptable, has no substitute. Moreover, the biological investigator, in wielding his formulae, should not remain content until the abstract "tendencies" or "concepts" he arrives at can be translated analytically into terms of the arrangements and methods of movement of concrete particles. In the opening up of the new fields in genetics, especially, a broad foundation in *all* the basic quantitative sciences—physics, chemistry and mathematics—will be increasingly important. The attainment of this ideal, however, will require a radical alteration in the educational equipment of biologists, and—if I may say so—in their genetic equipment. For of old natural selection was busy improving very different characteristics among biologists.

There remains one field of genetic study which I have not yet touched upon; it is the most recent to be subjected to a quantitative study, and at the same time, I believe, the most promising for the immediate future if attacked in this fashion. This is the field of gene mutation, which lies at the root of genetics even as genetics lies at the root of biology. A few years ago, to speak of studying mutation quantitatively would have seemed as absurd as to speak of measuring the frequency with which one finds a gold piece on the street, or a needle in a haystack. Each mutation was so rare as to be a unique event, and most of the experiments which purported to influence the course of genetic variation were really dealing with chance differences in the cropping out through segregation of re-

cessive genes previously "latent"; thus they were mistaking the straws for the needle. But so great has been the mass of data accumulated in the Drosophila work—where mutations are usually distinguishable from old segregates—that certain significant quantitative relations are already discernible there, and, though the data can not be used to ascertain the conditions under which mutation occurs, it can be used, as I have shown, in another way. By application of a formula to the respective numbers of loci that have mutated once, twice, three times, etc., it is possible to arrive at a *minimum* estimate of the total number of genes, including those that have not mutated at all, and so to arrive at a figure for the maximum possible size of the gene, which will become more accurate, and more significant for general physiology, as time goes on.

Still more recently, the problem of the structure of the gene has been attacked in another way, through the penetrating quantitative studies of Anderson, Eyster and Demerec, following on the prior work of Emerson. Here, in certain special genes, or genes in special phases, mutations are so frequent that they can be counted and measured with even greater ease than we can deal with crossovers in flies. And, as the changes in atoms, quantitatively analyzed, have given us pictures of their structure, so here this work appears to be resulting in a dissection of the genes in question into smaller elements—"gene-elements"—and the rearrangements of these elements may, it is hoped, be subjected to study even as before we studied the rearrangements of the genes as a whole within the chromosome. Elaborate numerical determinations and calculations are indispensable here. Now if, as Demerec's experiments have seemed to indicate, the existence of recombining elements within the gene is really a general phenomenon, we would have opening up here an entirely new and fertile field of quantitative ultra-genetics.

In the working of the field of mutation, it will not always be enough to wait for cases to happen for us and

then follow them up. The effect of varying conditions upon gene mutation, or upon gene-element mutation, must be measured, and here undoubtedly we tread on virgin and productive soil, despite the endless inconclusive and controversial literature on induced variation, produced outside the searchlight of factorial inquiry. But how can we obtain quantitative evidence concerning events ordinarily so rare, except in the above special cases of rapidly mutating genes? It would take too long here to explain the first attempts of Altenburg and the present author, in 1918 and 1919, to break this *impasse* through a study of lethal mutations—which, on theoretical grounds, I regarded as the most frequent of detectable mutations, and which, as the facts later showed, really do fulfill this condition. We met a very resistant obstruction in the difficulty of detection of lethals, but despite that we finally obtained a tentative figure for lethal mutation rate in the X-chromosome, and, further, an indication, but not a proof, that temperature may affect the rate of this mutation.

In the eight years since then I have constantly endeavored to improve these methods by the building up of special synthetic stocks. By the genetic mechanism of these stocks—which it would require too long a digression to explain—"natural selection" is automatically prevented, in such a way that the lethals are allowed to accumulate from generation to generation; the testing for lethals is done only at the end of a given number of generations, and thus minimized, and it is otherwise facilitated by the special genetic tools employed. With such stocks only partially perfected, an experiment on the effect of temperature was again tried by the author in 1919–1921, and another by Hanson and the author, three years ago, each time with inconclusive results, but results that pointed in the same direction. *"Carthago delenda est";* the walls of the rampart were shaking. The attack was renewed. And, in order that I may present you in this talk with at least one thing new, I wish here to announce to you that finally success has been

achieved. The experiments of the past year, on the second chromosome of Drosophila, when subjected to the tests for their probable error, show a really significant effect to have been produced. When we take all the experiments together, the proof is conclusive that, somehow, a moderate increase in temperature causes a decided increase in the rate of gene mutation in Drosophila—an increase of about the same magnitude as occurs in chemical reactions in general. Thus we see that gene mutation is not unreachable; it can be affected by temperature, even as chemical reactions are affected, and the effects can be measured. This being true, it is probably true also that other agents which affect chemical reactions can influence mutation likewise, and so a new field—that of the physiology of gene mutation—seems thrown open for quantitative study.

In conclusion, let us change our metaphor. We have seen how the female gametophyte of cytology has produced an egg, which, fertilized by sperm from the little male gametophyte of experimental breeding, formed a zygote called genetics. This zygote, inoculated with the growth-stimulating symbiotic virus of quantitative treatment, has been developing with a rapidity such that it has threatened to become a separate plant, overshadowing its parents. However, it has turned out that, remaining connected, instead of feeding upon or choking them to death, it is, by its own synthetic processes, forming a virile sap, that is beginning to be absorbed back into the parents and to sustain them as accessory structures. In thus combining to produce this remarkable new development, the female gametophyte has chiefly contributed those factors which make for the characteristic we have called "precise observation," the male, those for "manipulation," and the mathematical virus, those for "calculation." Now this young zygote is capable of mutating: watch it grow out new variegated branches; it is only at the start of its career. And I think I may add also that one of its component tissues—the Drosophila work—is only at the start of its career.

QUANTITATIVE METHODS OF RESEARCH AND THE PROBLEMS OF ANIMAL BREEDING[1]

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THE central problem of animal breeding, and for that matter all agriculture, was foretold more than 125 years ago in the conclusion of Malthus: "The power of population is indefinitely greater than the power of the earth to produce substance for man." Converted into rates against time, the natural antithesis of the two variables shows clearly that the object of animal breeding can be nothing less than to delay as long as possible the consummation of this prophecy by furnishing information to increase the production of more and better food per unit of its manufacture, the animal. This food must further be economically produced in the sense that the animal's consumption of carbohydrate, fat and protein in its roughage should bear as low a ratio as possible to the meat, milk or eggs which it forms. These are growth problems. To Francis Galton we owe not only the recognition of the need for, but also much of the technique by which a great deal of the progress in this solution is now taking place. Born a statistician he early began to enthusiastically collect statistics on problems in biology in much the same way that others collect postage stamps or beetles, but possibly with deeper design. This enthusiasm found direction in the unfolding Darwinian theories which appealed to him as subject to mathematical treatment and proof. For this purpose he collected all manner of data on such characters as human stature and

[1] Paper read at the Symposium on "Quantitative Biology" at the joint meeting of the American Society of Naturalists and the American Society of Zoologists, Philadelphia, December, 1926. From the Department of Animal Pathology of the Rockefeller Institute for Medical Research, Princeton, N. J.

intelligence. He evolved a novelty, now become a pest, a questionnaire. Prizes were offered for data when they came in too slowly. Until his work began no one appears to have had the idea to collect precise biological information capable of exact analysis.

De Moivre's early forgotten work, together with that of Laplace and of Gauss, had approached some of the problems necessary to the solution of such data in the shape of that demigod the "error curve or normal curve." This problem deals with one variable. The problem of two variables, related characters, had yet to be developed, as Galton soon found out. The coefficient he derived, with even its crudities of calculation, met this need and has remained with us under the title of coefficient of correlation. The finesse of the subject and its tremendous expansion came not so much through Galton's own efforts as those he inspired in others, notably, Weldon and Pearson. Weldon's work, the first quantitative approach to the problems of evolution, opened up more exact methods of calculation and gave science the idea of negative as well as that of positive correlation. This phase of the subject was soon carried much further by Pearson, to whom the world owes a great debt for developing an orderly analysis of frequency curves, goodness of fit criteria, the refinement and expansion of the correlation theory and other contributions of like magnitude.

But the recognition of the problem and developing the technique for it are not all, for the man making the opportunity for the investigator too deserves a real place in scientific progress. The far-sighted director of the Maine Agricultural Experiment Station, Dr. Charles D. Woods, was such a man. In 1897 he arranged this opportunity by establishing poultry work at the Maine station, the expressed idea of which was to analyze problems of animal breeding as related to the domestic fowl. The investigations progressed in the sense that valuable data were collected, but their solution did not appear. In retro-

spect it is now clear that they lacked the touch of the finished investigator commanding a thorough knowledge of the quantitative method. This contact was made in 1907 with the appointment of an outstanding exponent of quantitative biology, Dr. Raymond Pearl. From then on the experiments prospered, as an audience of American naturalists well knows, prospered to the extent that the general type and methodology of the investigation have been copied over and over again in the poultry investigations of others. To him, it also seems to me, the agriculturalist owes the firm place now held by quantitative biology in economic animal breeding.

The same two men, Pearl and Woods, soon foresaw the necessity for like problem analysis in the dairy cattle field. The opportunity was developed and research started in 1911. The inbreeding problem, a problem which up to that time had been thoroughly confused in the agricultural literature, first received attention. The first efforts were directed toward putting the problem on a quantitative basis, for the truth of Lord Kelvin's remark, "When you can measure what you are speaking about and express it in numbers, you know something about it, but when you can not measure it, when you can not express it in numbers, your knowledge is of a meager and unsatisfactory kind," was well recognized. The then vexing problem of inbreeding now seems simple, thanks to this work and that of Jennings, Robbins, Wright and others following it.

Three fundamentally important ideas have been brought to biology by the quantitative method. First, the measuring of a group of individuals in terms of the group's own attributes rather than those of the individuals; second, a means of correlating a series of characters; and third, the probable error concept as a method of testing the validity of experiments or comparisons.

The measuring of the group's attributes defines and differentiates the problem. Thus, as in Fig. 1, the milk production of Jersey cows for an eight months' lactation

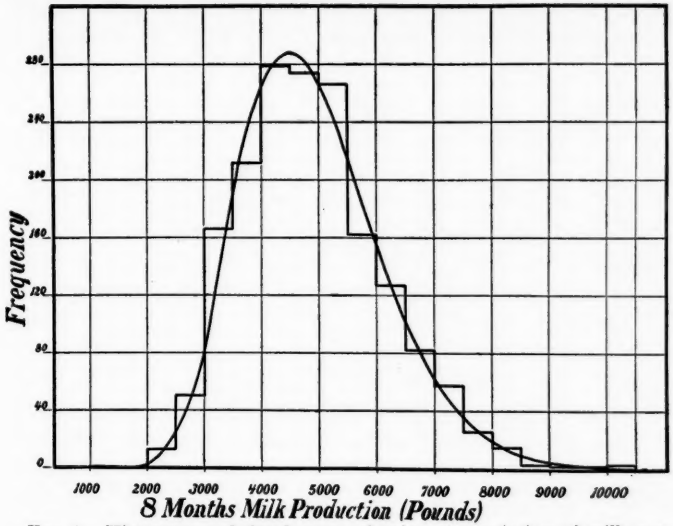

FIG. 1. Histogram and fitted curve, showing the variation of milk production in a purebred Jersey herd.

period has a characteristic curve of variation. This curve's constants furnish the taxonomic description of these cows in milk production. We know they are capable of a type or average milk secretion of 4,900 pounds—that the individual cows range in production from 1,000 to 10,500 pounds, with a standard deviation of 1,250 pounds. The frequency curve of production is significantly skew in the plus direction, $+ 0.32 \pm .02$. The most frequent or modal production is 4,500 pounds and the curve is of type I where the frequency curve for production is:

$$y = 288.7\left(1 + \frac{x}{3465}\right)^{8.09}\left(1 - \frac{x}{91385}\right)^{213.3}$$

This curve is a composite of those of lactations at different ages. The analysis of these individual curves, Fig. 2, shows that the curves are unimodal, tend to be less skew than the total production curve and show a uniform variation in both their means and standard deviations

when these are related to age. The function relating milk secretion to age is shown in Fig. 3 for the mean milk yield, and in Fig. 4 for the standard deviation. Through a knowledge of these relations it is possible to eliminate more than 10 per cent. of the original variation in milk yield by correcting for the age influence in much the same way the physicist corrects his readings for atmospheric conditions; or in the analytical sense, growth changes cause a marked variation in yield, the law relating milk yield to age and variation of milk yield to age being shown by the smooth lines on the charts.

After the rediscovery of Mendel's law it became evident that the concept held by Darwin and Galton clashed in many particulars with that held by Mendel with regard to the physiological processes behind inheritance. It came to be thought by many investigators that the quantitative Mendelian principles rendered futile the older methods of the biometric school. The majority, in fact, believed that the essential problem was clouded rather than clarified by the application of such methods and blinded themselves to all such work. The growth of

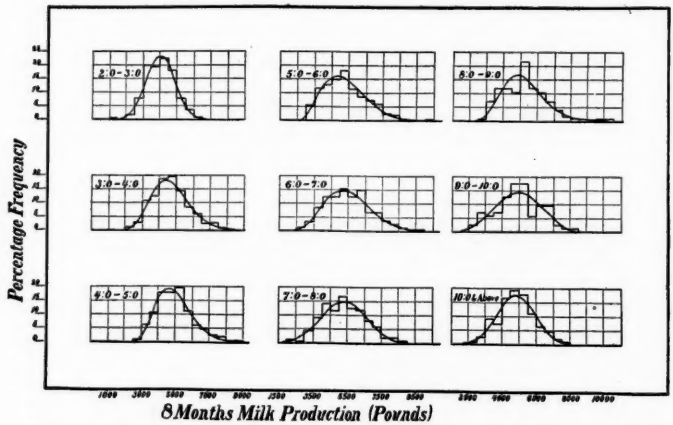

FIG. 2. Histograms and fitted curves, showing the variation of milk production for each year of age. The area of each curve reduced to unity to facilitate comparison.

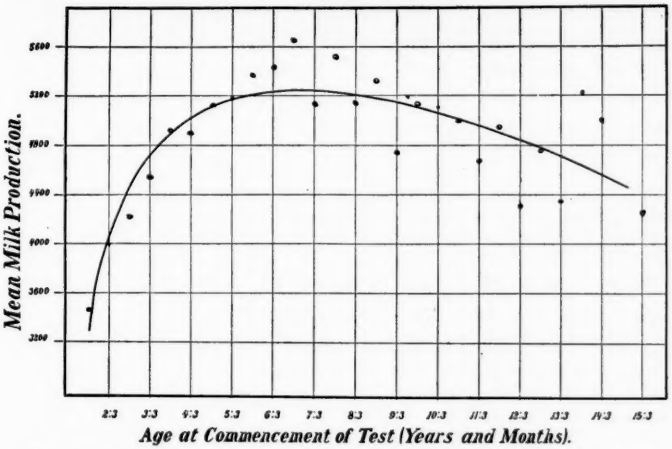

FIG. 3. Diagram showing the observed means and theoretical means of Jersey milk production at any given age.

Mendelism, as typified by papers by Pearson, Johannsen, Wright and many others, has rendered judgment against this attitude, for it has now come to be recognized that biometric methods are essentially applicable to Mendelian problems and become especially valuable in those dealing with characters caused by multiple factors in slow breeding animals. The economic animal breeding problems are predominantly of this type. In fact, the problems of animal breeding are of such a complex nature that they may be pictured as due to a multiplicity of causes, each bearing upon and building up the variability of the character studied. Milk yield is such a variable. The factors which bring about these differences in production may be grouped under the general titles of changing environment, permanent environment, and heredity. The quantitative method as a means of analyzing such problems may be illustrated by an analysis of the genetic influence on milk yield. The records chosen are those of the Holstein-Friesian breed corrected to a standard age of eight years and three months. The correlations of the different primary ancestral combinations

are shown in Fig. 5. Those familiar with the demands of a multiple factor population with slight assortative mating will recognize the close approximation of these results to those demanded by Mendelian theory. Three widespread applications to inheritance problems are illustrated—the comparison of the somatic resemblance of the ancestors with the offspring and its relation to Mendelian inheritance; the assortative mating within the inheritance; and the separation and measurement of the interacting causes of variation within a universe, in this case particularly environment and heredity.

Like all sciences, the ultimate aim of biology is the prediction of future events from known facts. Quantitative biology, as applied to either physiology or genetics, furnishes such criteria. In formulating such a prediction for Guernsey cows' milk yield it was found that the following relation existed between the milk yields of a cow, her sire's progeny performance test, and her dam's milk yield:

Daughter's milk yield = 1767 + .60 sire's progeny performance + .25 dam's milk yield.

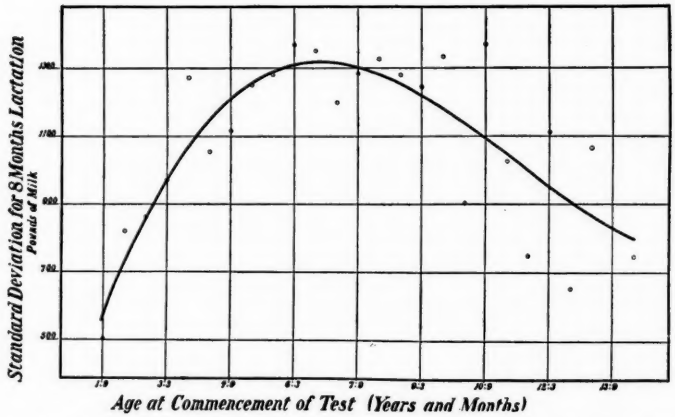

FIG. 4. Observed and theoretical standard deviations of milk production at successive years.

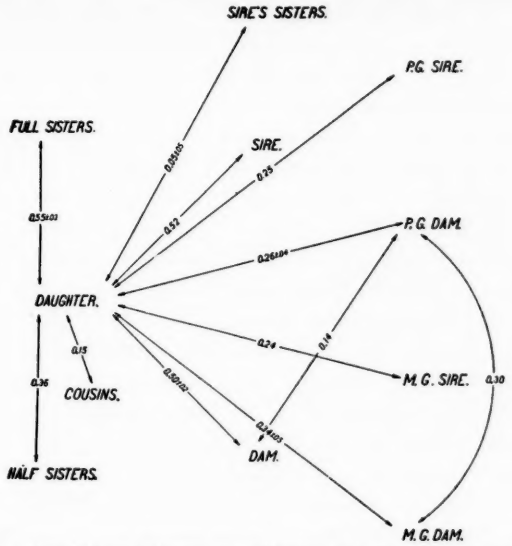

FIG. 5. Correlations between the offspring and the first and second generation ancestors for Holstein-Friesian Advanced Registry cows.

The application of this prediction to 1,082 Guernsey cows which have sires with progeny performance records and dams of known milk yield is shown in the following table. The average daughters' actual milk yields agree fairly well with those which are predicted by means of the available facts. Thus when the daughters' milk yield is predicted to be 11,000 pounds, it is found that the daughters' average production actually is 11,317 pounds. Where the daughters' production is predicted to be 11,500 pounds, it actually is 11,640 pounds on the average. These predictions are fairly close. There are other differences which are as much as 1,500 pounds, however. Such examples occur in the groups made up of few cows where the error is greater.

It is clear that by no means all the variation between predicted and actual records is accounted for when information on the sire's progeny performance and the dam's milk yield is alone available. The quantitative

method gives a certain feeling of security, however, in that it is possible to determine the amount of variation which should actually be eliminated. In the case under discussion the theoretical total correlation between the cow's actual record and her predicted record should be 0.48. In the prediction as made the actual correlation is $0.55 \pm .02$.

But the bell-wethers of biology, experiment and observation, have they no debt to the quantitative development? The history of experimentation shows that they have. The study of the mathematics of variability early showed beyond peradventure that every systematic error and unknown contributing cause should be eliminated or they become a menace to the conclusion. No mass collection of data will ever overcome this need. The philosophy of experimentation has likewise been markedly changed by the introduction of quantitative methods. Thought is now given not only to the technique of the experiment but also to the technique of the analysis of the results which are hoped to accrue therefrom. It has

Correlation between daughter's predicted milk yield and daughter's actual milk yield, Guernsey Advanced Registry cattle

Daughter's predicted milk yield	Daughter's actual milk yield: 6000–7000	7000	8000	9000	10000	11000	12000	13000	14000	15000	16000	17000	18000	19000	20000	21000	22000–23000	Total	Daughter's mean actual yield
8267–8767	4	1	2	3														10	7900
8767		13	6	3	1													23	8150
9267	1	7	7	12	12	5	1	1										46	9610
9767		16	21	22	13	10	7		2	1	1							93	9760
10267	3	5	22	27	28	31	17	9	16	2	2							162	10990
10767		4	9	39	51	44	21	24	6	5	3	2	2					210	11370
11267		1	10	22	36	32	23	25	10	3	2	1	1					166	11640
11767		1	7	9	18	20	26	18	13	8	4	1	2					127	12380
12267		2	2	5	11	12	19	17	6	5	6	1	1	1				88	12720
12767				4	6	13	12	18	10	4	7	5		3	1		1	84	13750
13267			1		2	3	5	9	10	7	2	3	3	1				46	14390
13767					2		1	6	3	2	2	2	1		1	1		21	15100
14267			1			1							1	1				4	14500
14767																			
15267–15767								1	1									2	14000
Total	8	50	88	146	180	171	132	128	77	37	29	15	11	6	2	1	1	1082	

TABLE 1. Correlation between daughter's predicted milk yield and daughter's actual milk yield, Guernsey Advanced Registry cattle.

come to be realized that in its ideal the elements which are varied under experimental control should be uncorrelated. The influence of these facts has become increasingly manifest in the tendency of all science toward exactitude in plan, technique and analysis.

By beginning with the historical introduction of quantitative methods into animal breeding and proceeding with illustrative material, I have attempted to picture the trend of biology. This trend, I believe, shows that the day is past when arguments for quantitative research are in any way helpful. Whether we wish it or not, biology is surely being put on a quantitative basis in just as irresistible a manner as physics and chemistry, which preceded it slightly in time. The younger proselytes to the science will receive even shorter hearings unless they describe in terms of magnitude and its probable error, and their experimental proofs are stated in terms of odds. This coalition of mathematics and biology is here to stay because it greatly increases our analytical acumen and augments the achievements in the penetration of the unknown phenomenon of life.

THE CONVERGENT IMPROVEMENT OF SELFED LINES OF CORN

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INTRODUCTION

SELECTION within self-fertilized lines has become the accepted principle in breeding corn to obtain yields materially larger than those which can be had from the best of the present open-pollinated varieties. The procedure is essentially that outlined by Shull (1909) in his original suggestion for "A Pure-line Method in Corn Breeding." Numerous plants are self-pollinated, and seed of the resulting ears is planted an ear to the row. Plants in these rows again are self-pollinated and the selfed ears from the better plants in the better rows are selected for continuing the breeding program. Many of the selfed lines are so poor as to be worthless. Others are obtained which are reasonably normal and can be propagated readily. No selfed lines superior to the parent stock have been reported. Their value for obtaining larger yields of corn lies in their use in hybrid combination of one kind or another.

At least three methods for utilizing selfed lines in practical corn production are being compared. Two of these, the use of F_1 crosses between two lines (Shull, 1909), and the use of double crosses, or F_1 crosses between two F_1 crosses which involve different lines (Jones, 1918), utilize the maximum of hybrid vigor available only in the F_1 generation. Their operation consequently requires that the parent lines be maintained and that F_1 seed be produced anew each year. This necessitates growing seed of two selfed lines and one cross if F_1 crosses are used, and of four selfed lines and three

crosses if double crosses are used. The third method being investigated is to intercross several selfed lines which are known to produce the desired characteristic in all combinations, and then to continue this mixture of crosses by mass selection under open pollination (Emerson and East, 1913) as a synthetic variety (Hayes and Garber, 1919). This method obviates the necessity for growing crossed seed each year.

Data on the yields obtained experimentally have been reviewed recently (Hayes, 1926; Richey, 1927). For the present purpose it is sufficient to note that significant increases have been obtained by each method and that data are not available for comparing the methods. Which will prove most profitable will depend both upon its efficacy in obtaining larger yields and upon the practicability of seed production and distribution under it. Thus, double crosses must be more productive than single crosses or must effect a material saving in the final cost of the seed produced if they are to compensate for the greater difficulty of maintaining twice as many parent lines and making three times as many crosses. It is in its practicability that the synthetic variety has an important advantage. It seems improbable that a mixture of many crosses can be obtained which will give as large a yield as the best of the component crosses in the F_1 generation. The productiveness of such a mixture in the later generations, after segregation and recombination of the genetic factors has had time to occur, is even more problematic. Nevertheless, the practical advantages of seed production and distribution which this method offers may be more than sufficient to balance the somewhat smaller yields which seem probable.

The necessity for utilizing hybrid combinations would be avoided if selfed lines yielding as much as present hybrids could be obtained, and the whole problem of seed production thereby would be simplified. Regardless of this possibility, however, and of the methods of hybridization to be used, it evidently is desirable to have selfed

lines that are productive in themselves. Two possibilities of obtaining higher-yielding selfed lines have been recognized generally: (1) obtaining them by more extensive selection within selfed lines in ordinary varieties, and (2) obtaining them by selecting within selfed lines from crosses between the best lines previously isolated.

Under either of these methods nothing can be predicted definitely as to the behavior of the recovered lines in hybrid combination. The poorer lines have produced the higher yielding crosses in some experiments. It therefore would be very desirable to be able to increase the productiveness of the better selfed lines already isolated while insuring against the loss of those factors which cause them to produce high-yielding crosses. The possibility of achieving this is afforded by a system of crossing two lines, pollinating back to the parents, and again selecting within selfed lines. It is the purpose of the present paper to consider this method and some of the conditions which will determine its success or failure in practical corn improvement. Analysis of the causes of such success or failure in breeding experiments should give additional information as to the basis of hybrid vigor and as to the elements determining the total absolute productiveness of F_1 crosses between selfed lines of corn.

THE PROBLEM

To simplify the discussion it is desirable to assume for the moment that the vigor and productiveness of F_1 crosses between selfed lines of corn is a direct function of the number of expressed genetic factors favorable to development in the given environment. It also is convenient to assume that these factors are equally important, that dominance is complete in all the factor pairs concerned, and that there is no linkage between the different factors. It is not intended to postulate the truth of these assumptions. Their temporary acceptance will obviate the need for the incessant repetition of provisional exceptions. Linkage, unequal importance of the

factors, partial dominance, and physiologic stimulation as a possible partial cause of heterosis will be considered later.

Under these assumptions, the objective of the corn breeder seeking to obtain a higher yielding line that will maintain itself under self-fertilization becomes merely to concentrate the necessary favorable factors in the homozygous condition within this line. The difficulty lies only in the large number of factors presumably needed to attain this objective, together with the small effect of the individual factors. In view of this objective, the importance of selecting the best foundation stock need not be emphasized. Not only should the stocks carry as many favorable factors as possible, but they should be sufficiently homozygous to constitute quite definite entities.

Two pairs of selfed lines isolated in the course of the writer's breeding experiments with Delta Prolific corn (Cereal Investigations No. 201) may be utilized to give a more concrete idea of the problem and how the method may be employed. In the pedigrees of these lines which follow, each of the numbers separated by dashes indicates a generation of self-fertilization. The S_2 and S_3 indicate two and three generations of self-fertilization after detailed pedigrees were discontinued.

B. 2-2-2-4-2-1-S_2
C. 5-1-2-2-1-3-1-2-S_3
F. 7-4-2-1-1-2-1-2-S_3
H. 10-3-1-1-2-2-1-2-S_3

Using the index letters to the left, lines C, F, and H have been selfed for eleven generations and line B for eight generations. All, therefore, are practically homozygous.

The relative yields of crosses between these lines reported previously (Richey, 1924; Richey and Mayer, 1925) have been confirmed substantially in more recent experiments. Of the six possible combinations between these lines, disregarding reciprocals, only the combination BF has failed to give a larger yield than that of the parent variety, one of the better varieties for the region of the experiments. Furthermore, lines C, F and H tend

to combine well with other unrelated lines, although this tendency is marked only in line H. Finally, the double cross BF × CH has produced yields materially in excess of those of the parent variety. These lines, therefore, apparently carry at least a large proportion of the important dominant factors necessary for yield, and are alike for such necessary factors as are recessive.

That each line lacks certain important factors is shown by the fact that the selfed lines are so poor as to make production of the single-crossed seed difficult. This seriously limits the usefulness of the lines in the practical production of seed corn. The desirability of increasing the vigor and productiveness of these lines without interfering with their performance in hybrid combination is obvious.

The factors for which lines B, C, F and H are alike may be disregarded. These will include any important recessive factors and some, presumably many, favorable dominant factors. With the use of the double cross, BF × CH, as the ultimate objective, and considering first only the improvement of a single line, the problem then may be stated more specifically as being to transfer to line B the dominant favorable factors which it lacks, but which are carried by line F, at the same time maintaining that part of the original B genotype that causes this line to yield well in hybrid combination.

IMPROVING A SINGLE LINE

Plants of the F_1 cross between lines B and F, and of the resulting progenies, are pollinated with pollen from line B through several generations. During this period of back pollinating, however, seed ears are selected from only the most vigorous and productive plants. Moreover, after the first generation of back pollinating, the seed is planted in ear rows to permit selecting from the better progenies as well as from the better individuals. If an isolated plot is available, line B may be planted in every third or fourth row to furnish pollen, the plants in

all other rows being detasseled. This will provide a maximum population with a minimum of labor, and the yields of the ear rows will not be modified by the effects of hand pollinating and so will offer a better quantitative basis for selection. Replication may be provided by planting the seed from each ear in several shorter rows rather than in one longer row. Inability to provide an isolated plot will increase the labor by necessitating hand pollinating. Otherwise much the same procedure may be followed.

This practice involves two principles, (1) pollinating back to line B to insure recovery of the dominant favorable factors coming into the original cross from this line, and (2) selecting seed from the most vigorous, productive progenies and plants in order to retain the dominant favorable factors coming into the original cross from line F. Such of the latter factors as may be retained will be kept heterozygous as long as back pollinating is continued, inasmuch as their recessive allelomorphs are brought in from line B in each generation. Consequently, it is necessary to follow the period of back pollinating with a period of selection within selfed lines to obtain as many as possible of the F factors in the homozygous condition.

The recovered line, which may be designated B(F′), will be homozygous for the favorable dominant factors of the recurrent parent, line B. Its value in crosses accordingly should be much the same as that of this line. At the same time line B(F′) will carry as many favorable dominant F factors as selection has been effective in retaining. To this extent it will be more vigorous and productive than line B.

Recovering the Genotype of the Recurrent Parent

The operation of back pollinating in insuring recovery of the dominant B genotype needs only brief consideration. One half of the gametes in each generation of back pollinating will be contributed by line B. Furthermore, as line B is homozygous, the B gametes will all be alike

and, without selection, the percentage of homozygosity will increase in successive generations according to the series ½, ¾, ⅞, etc. (Jennings, 1916; Wright, 1921). The percentage of homozygosis for the populations in successive generations, when no selection is practiced, is shown by the first line of values in Table I, being the same as the percentage of individuals that are homozygous when only one factor pair is concerned. The desirable factors coming in from line B are dominant. They therefore will be expressed in each generation and so will afford no basis for selection. The increase in homozygosis for these factors consequently should proceed according to theory. The percentages of individuals that should be homozygous for all the dominant B factors in different generations are shown for different numbers of factors in Table I.

TABLE I

PERCENTAGES OF PLANTS HOMOZYGOUS FOR THE n FACTORS ENTERING A CROSS ONLY FROM THE HOMOZYGOUS PARENT TO WHICH THE CROSS AND THE RESULTING PROGENIES ARE MATED IN EACH OF r SUCCESSIVE GENERATIONS[a]

$$\left[\frac{(2^r-1)^n}{2^{rn}}\right]$$

Number of factor pairs n	Numbers of generations of back pollinating (r)									
	1	2	3	4	5	6	7	8	9	10
[b]1	50	75	88	94	97	98	99	100 –	100 –	100 –
5	3	24	51	72	85	92	96	98	99	100 –
10		6	26	52	73	85	92	96	98	99
15		1	13	38	62	79	89	94	97	99
20			7	28	53	73	85	92	96	98
30			2	14	39	62	79	89	94	97
40				8	28	53	73	86	92	96
50				4	20	46	68	82	91	95
75					9	31	56	75	86	93
100					4	21	46	68	82	91

[a] Subject to errors incident to the use of 6-place logarithms.

[b] The values for $n = 1$ give also the percentages of homozygous factor pairs in the entire population regardless of the value of n.

The number of generations through which back pollinating must be continued is problematic and will differ

with the objective of the program. The probability that the recovered line B(F′) may be used in F_1 crosses, in place of line B, will depend upon the probability that it carries all the dominant favorable factors which have made line B an outstanding parent. After six generations, 46 per cent. of the plants would be homozygous for as many as 50 factors entering the cross from line B and only about 4 per cent. of the 50 factor pairs would be heterozygous in the other 54 per cent. of the population. The probability of recovering the important part of the B genotype after six generations of back pollinating accordingly seems adequate for purposes of practical corn improvement. A greater probability that the B factors will be retained in the recovered line will be had after ten generations of back pollinating. Then, 91 per cent. of all plants would be homozygous for 100 B factors and 75 per cent. would be homozygous for as many as 300 B factors. This might be a more adequate basis for theoretical investigations of the cause of hybrid vigor.

It is desirable to distinguish again between the two concepts involved. One of these postulates the recovery of the favorable part of the B genotype. Any definition of the number of generations necessary to accomplish this must assume some approximation as to the number of factors involved. The ultimate accomplishment, however, involves no assumptions other than an absence of complete linkage between one or more desired B factors and the recessive allelomorphs of favorable F factors to be retained by selection. Such linkage would prevent combining the two dominants by any method. It is the certainty of retaining the favorable part of the genotype of the recurrent parent by back pollinating that recommends its practice. By itself, back pollinating is powerless to effect improvement. This must be accomplished by selection.[1]

[1] An interesting discussion of the difference between the rôles of back mating and other kinds of inbreeding in animal improvement, by Muller (1926), recently has been published.

Selection in Back-Pollinated Lines

The improvement must come through retaining favorable F factors by selection, both during the period of pollinating back to line B and during the subsequent period of self-fertilization. The extent to which this can be accomplished will depend upon the total number of F factors involved and the number necessary to produce obvious differences as a basis for selection. Line B has yielded around 20 bushels per acre under reasonably good conditions when the cross between lines B and F yielded around 60 bushels. Under less favorable conditions, both lines B and F have been completely barren when the cross yielded about 40 bushels. In the presence of the favorable factors from line B, therefore, a difference in acre yield of about 40 bushels may be attributed to the action of the favorable factors from line F.

The adequacy of this in providing a selection differential will depend upon the number of factors responsible. Collins (1921) has shown that with as few as 20 equally important factor pairs heterozygous in the F_1, the distribution to be expected under ordinary experimental conditions in the F_2 would be indistinguishable in type from those obtained in practice. This may be taken as an approximation of the minimum by which lines B and F differ, with each line contributing about half, or 10 dominant factors. Possibly the upper limit is infinite. For present purposes it may be placed at 80 factor pairs with 40 dominant factors entering the cross through each line. This number is used because it probably is larger than the maximum that would permit material influence by selection in back-pollinated lines with a difference of 40 bushels in acre yields.

Any consideration of what may be accomplished by selection under such assumptions must be highly speculative. Nevertheless, it affords a basis for comparing the possibilities under different hypotheses. All the genetic variation in yield among the plants of any progeny in any generation will be the result of the various

combinations of dominant F factors in the gametes of the pistillate (back-pollinated) parent plant. The plants grown from the kernels on any ear accordingly will be distributed, as regards genetic variation, in accordance with the expanded binomial $(1+1)^n$, where n is the number of dominant F factors carried by the pistillate parent plant. The possibility of retaining these dominant F factors by selection during back pollinating will depend, first, upon the probable frequency of occurrence of plants carrying the larger numbers of F factors in populations that can be grown practically, and, second, upon the ability to determine these plants when they do occur.

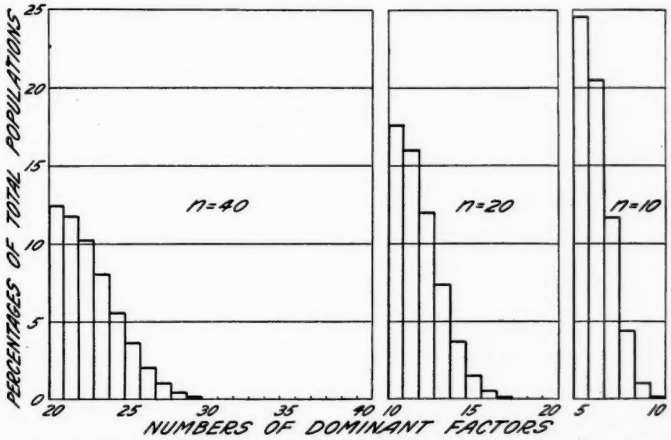

FIG. 1. The percentage frequency distributions for the upper $(n/2)+1$ classes for numbers of dominant factors in populations from individuals heterozygous for n factor pairs mated to individuals homozygous for the n recessive allelomorphs.

The percentage distributions of plants in the upper $(n/2)+1$ classes, with n equal to 40, 20, and 10, respectively, are shown in Fig. 1. It is obvious that the frequencies of individuals with the maximum numbers of F factors are so low as to preclude probability of the regular occurrence of such individuals in ordinary populations. Even when $n=10$, only 1 plant in 1,024 would

carry all 10 F factors. For values of n greater than 10 there would be opportunity to select plants having only a very few factors in excess of the number carried by the midclass. With $n = 5$, however, 1 plant in 32 should carry all 5 factors and it should be possible to retain this many indefinitely, provided plants carrying them can be recognized with reasonable assurance.

For the higher values of n, selection during the earlier generations of back pollinating would be effective mainly in insuring that at least half of the F factors were retained. Selection would have an increasing influence as the value of n decreased in successive generations. In so far as numerical relations are concerned, it would seem entirely possible to retain at least 5 of the dominant F factors, regardless of the number coming into the cross originally.

The possibility of recognizing the plants with the larger numbers of F factors would be influenced more strongly by the total number effecting the increase of 40 bushels per acre when lines B and F are crossed. With a stand of 7,000 plants per acre this is equivalent to a difference in plant yield of .40 of a pound, or to 40 pounds per 100-plant row. With 40 factors of equal importance, each would add .01 of a pound, or 5 per cent., to the basic plant yield of line B.

This probably is about the smallest difference which could be determined with sufficient frequency to give a reasonable probability of determining individuals carrying 5 F factors. It may be too small. The percentage distributions for the $(n/2) + 1$ classes having the larger numbers of dominant F factors were shown graphically in Fig. 1. The percentages of the entire populations included within enough of these extreme classes to provide totals comprising from 6 to 8 per cent. when $n = 40$, 20, and 10, are shown in Table II. The weighted average acre yields of these more productive classes when the n factors determine a difference of 40 bushels also are given, together with the acre yields of the classes having the next lower numbers of dominant F factors. Thus, when $n = 40$, the difference in acre yield between the most

productive 7.69 per cent. of the population, having from 25 to 40 dominant F factors, and the 5.72 per cent. of the population having 24 dominant F factors, would be 1.9 bushels. With fewer F factors responsible for the total difference, the assurance of identifying individuals carrying them increases. With approximately 40 or fewer F factors involved, it is possible that lines can be recovered which would be heterozygous for some 5 dominant favorable factors entering the original cross from line F.

TABLE II

THE AVERAGE ACRE YIELDS OF THE CLASSES COMPRISING THE UPPER 6 TO 8 PER CENT., AND OF THE NEXT LOWER CLASSES, IN THE POPULATIONS SHOWN IN FIG. 1.

When the total number of factors determining an acre difference of 40 bushels is:								
40			20			10		
Classes for dominant factors	Per cent. of population	Yield	Classes for dominant factors	Per cent. of population	Yield	Classes for dominant factors	Per cent. of population	Yield
27–40	1.92	47.7	15–20	2.07	50.8	8–10	5.47	52.9
26	2.11	46.0	14	3.70	48.0			
25	3.66	45.0						
25–40	7.69	45.9	14–20	5.77	49.0	8–10	5.47	52.9
24	5.72	44.0	13	7.39	46.0	7	11.72	48.0

Selection in Selfed Lines

Under the same conditions it should be relatively easy to recover a selfed line homozygous for the 5 F factors when back pollinating to line B is terminated. The minimum number of plants necessary to provide for the entire expected distribution is 4^n, among which the number carrying all of the dominant factors is 3^n, n being the number of factor pairs heterozygous in the parent. Thus, 243 of every 1,024 plants, or about a quarter of the population, would be either homozygous dominant or heterozygous for all of 5 factor pairs for which the parent was heterozygous. The percentage frequency distribution of these 243 plants with reference to the heterozygous condition for different numbers of factors is shown in Table III. If progress in obtaining the homozygous dominant condition can be made in each generation, a line homozygous

for the 5 dominant F factors will be obtained in the F_6 generation. The percentage frequency distributions shown for different values of n in Table III indicate the probability of even more rapid progress, provided the plants carrying the dominant factors can be determined with reasonable assurance.

TABLE III

TOTAL POPULATIONS NEEDED TO PROVIDE FOR THE ENTIRE GENOTYPIC DISTRIBUTION FOLLOWING SELF-FERTILIZATION WHEN THE PARENT WAS HETEROZYGOUS FOR FROM 1 TO 5 (n) FACTOR PAIRS, THE NUMBERS AND PERCENTAGES OF INDIVIDUALS COMPLETELY DOMINANT PHENOTYPICALLY, AND THE PERCENTAGES OF THESE INDIVIDUALS THAT ARE HETEROZYGOUS FOR VARIOUS NUMBERS OF FACTOR PAIRS.

	Total population	Individuals phenotypically dominant		Percentages of phenotypically dominant individuals which are heterozygous for the following numbers of factors					
n		No.	Per cent.	5	4	3	2	1	0
5	1,024	243	23.7	13.2	32.9	32.9	16.5	4.1	0.4
4	256	81	31.6	.....	19.8	39.5	29.6	9.9	1.2
3	64	27	42.2	.....	.....	29.6	44.4	22.2	3.7
2	16	9	56.3	.....	.....	.....	44.4	44.4	11.1
1	4	3	75.0	.....	.....	.....	.....	66.7	33.3

On this basis it should be entirely possible to obtain a homozygous line B(F′) within 12 generations of back pollinating and selfing, equal to line B for use in crosses and superior to line B because of the dominant F factors added. Moreover, the number of dominant F factors carried by line B(F′) should approximate 5 when line F contributed from 10 to perhaps 40 dominant favorable factors to the cross.

The speculative background for such a conclusion is recognized clearly. This is a necessary concomitant of the scarcity of data bearing on the subject. At the same time, every effort has been made to be conservative. Moreover, the considerations leading to this conclusion constitute an attempt to examine some of the quantitative relations involved in determining the probability of success, rather than a demonstration of such probability. The final assumption of 5 F factors in line B(F′) affords merely a convenient quantitative basis for the later discussion.

Convergent Improvement

The factorial relations will be similar to those already considered if the cross between lines B and F is mated back to line F. Here, however, the recovered line F(B′) will be homozygous for all the dominant favorable factors of line F and for some 5 from line B. Inasmuch as the selection of line B(F′) may proceed synchronously with that of line F(B′), both of these lines should be obtained within the time required to grow 12 generations. Similarly, lines C(H′) and H(C′) can be produced at the same time.

Lines B(F′) and F(B′) will differ by some 10 fewer dominant favorable factors than do lines B and F. Moreover, as the procedure outlined is concerned only with those factors by which the parent lines differ, it can be followed again with lines B(F′) and F(B′) as parents, just as effectively as before. In this way lines with two increments of added factors, B(F‴) and F(B″) may be obtained, and so on. With an initial difference of 80 factors, reduction by 10 at a time would require 8 repetitions of the program to produce a line homozygous for all of the dominant B and F factors. With an initial difference of 20 factors, the same result would be accomplished in 2 repetitions of the improvement program, requiring some 24 generations.

In either case, the final resulting line would be as productive as the F_1 cross between lines B and F and should maintain this productiveness indefinitely. The degree to which such a line can be approximated will determine the value of the method in practice. The development of such a line, or the determination of the causes preventing such development, should add to an understanding of the principles involved.

Practical Possibilities

The preceding discussion was concerned with hypothetical factors causing equal increments of vigor and productiveness in order to provide a basis for examining the quantitative relations involved. The probability of obtaining practical advantages from applying the prin-

ciple of convergent improvement rests upon a more concrete foundation. It is not essential that a homozygous line yielding as much as the F_1 cross between the parents be developed. Any material increase in the productiveness of the recovered lines that can be obtained without lessening their value in hybrids will be worth while. Such lines could be used more profitably either in producing single-crossed or double-crossed seed or in synthetic varieties as conditions dictated.

At least some of the unfavorable recessives in the foundation lines, which are to be replaced by their favorable dominant allelomorphs, cause obvious defects which constitute a perfectly definite basis for selection. Numerous deficiencies appear during the earlier generations of selection within selfed lines and homozygosis is approached so rapidly that many become fixed, some even before they are recognized as deleterious. The principle of convergent improvement offers the most definite and certain means of eliminating such defects without interfering with the behavior of the lines in hybrid combination.

Lines free from these defects doubtless could be recovered by selecting within selfed lines immediately after crossing. Such a procedure, however, would necessitate selecting simultaneously to eliminate the deleterious factors from both parents, thereby greatly increasing the complexity of the selection program. The initial period of back pollinating, during which the desirable characters of the recurrent parent are fixed, substitutes an additional time element for this increased complication.

Of far more importance is the rôle of back pollinating in fixing those factors which have caused the parent lines to yield well in hybrid combinations. Lines recovered by selfing immediately following the original cross must be tested in various combinations to find out which, if any, will combine to advantage. This involves a period of experimentation which may well exceed the period devoted to back pollinating in time and cost. Moreover, although immediate selfing may provide better opportu-

nity for obtaining crosses which will yield more than the crosses between the original parent lines, it also involves the possibility of loss in this respect. Selection within selfed lines immediately following crossing, therefore, does not provide the definite assurance of improving the productiveness of the selfed lines without interfering with their value in hybrids that is offered by the principle of convergent improvement.

Theoretical Considerations

It was assumed earlier, as a working hypothesis, that the vigor and productiveness of F_1 crosses between selfed lines of corn was a direct function of the number of expressed genetic factors favorable to development in the given environment, and that these factors were completely dominant, of equal importance and independent in inheritance. Obviously, the factors concerned with vigor and yield are not of equal importance, and probably some of them are linked.

It is the inequality of effect among the factors that provides a concrete basis for applying the principle of convergent improvement to practical corn breeding with much hope of success. At the same time this condition tends to inhibit the attainment of homozygous selfed lines as productive as the F_1 cross. With some factors known to have an important effect, the average influence of the remaining factors must be smaller, and less opportunity is provided for selection among these residual factors. The quantitative relations for different numbers of factors have been considered briefly and further speculation would be unprofitable. Experiments on improvement by the method outlined should provide some information on this question.

It is probable that linkage occurs among some of the factors affecting vigor and productiveness in corn, as it does among some of the factors the mode of transmission of which has been determined. Linkage between favorable factors would be beneficial to the program of im-

provement. Linkage between favorable and unfavorable factors, on the other hand, would delay progress. Success in obtaining any desired combination would be inhibited only if the factors concerned were linked so closely that no crossovers could be obtained. This would be equally inhibitive under any other method of breeding and, short of this, any given linkage would interfere less under back pollinating than under selection within selfed lines.

The principle of convergent improvement necessarily is concerned only with favorable factors which are completely or partly dominant, and which therefore can be recognized in the heterozygous condition. Back pollinating may be utilized of course to transfer a desired recessive factor from one selfed line of corn to another. Occasional selfing will be necessary in such cases to bring the factor into expression. The procedure will be essentially that outlined by Harlan and Pope (1922) for producing a smooth-awned barley, in exemplifying the use of back pollinating in small-grain breeding. Its operation will be facilitated by the simplicity of controlling pollination in corn. The corn breeder working with selfed lines which yield well in hybrid combination, however, is concerned with dominant factors. It is this fact, together with the simplicity of controlled pollination, that permits selection in back-pollinated lines and suggests the principle of convergent improvement.

The possibility of partial dominance is of interest in another way. With favorable factors which are only partially dominant added to the parent lines, crosses between the recovered lines should yield more than the crosses between the original parent lines. Thus, it is possible that convergent improvement may result in increased yields of the crosses as well as of the parent lines. Here again experiments are needed before definite statements can be made.

Hybrid Vigor

It is in connection with the cause of hybrid vigor that experiments on convergent improvement in corn have

their greatest interest. There can be no question that much of the decrease in vigor accompanying inbreeding and the increase following crossing are the effects of the expression of deleterious recessive factors and favorable dominant factors, respectively. Some of these effects are obvious. Others are more elusive, but nevertheless clear to those familiar with the idiosyncrasies of their breeding stocks. It is entirely reasonable to assume that all hybrid vigor may be explained on the same basis. At the same time it has not been proven that this is so, and the possibility of physiologic stimulation due to the presence of heterogeneous germplasm within the cells is one of great interest and importance.

Experiments on convergent improvement of selfed lines of corn should provide critical evidence on this question. With each successive step in the improvement program the recovered lines will be more nearly alike genetically than their parent lines, provided their yields in the homozygous condition are larger than those of the parent lines. Under the hypothesis of physiologic stimulation, the yields of crosses between the recovered lines should tend to decrease with this increasing similarity of germplasm in the parents. Under the hypothesis of the complementary action of dominant favorable factors, on the other hand, the yields of crosses between the recovered lines should equal those of crosses between the parent lines. With incomplete dominance of the factors concerned, the crosses between the recovered lines should be even more vigorous and productive. Consistent tendencies in one direction or the other accordingly should furnish additional support for one of the hypotheses.

Summary

The need of a method for improving the productiveness of selfed lines of corn without interfering with their behavior in hybrid combination is pointed out, and the principle of convergent improvement is suggested as meeting this need.

Convergent improvement involves the reciprocal addition to each of two homozygous selfed lines, which combine to produce a high yielding cross, of those dominant favorable factors which one line lacks, but which are carried by the other.

Convergent improvement involves: (1) crossing the two lines, (2) back pollinating to one line through each of several generations to recover the genotype of the recurrent parent, (3) at the same time practicing selection to retain favorable factors entering the cross from the other parent, (4) selecting within selfed lines to fix the added factors in the homozygous condition, (5) performing these operations in parallel with each of the original lines as the recurrent parent, and (6) repeating the operation to achieve further improvement, using the recovered lines in place of the original parent lines as foundation stocks.

It is concluded that convergent improvement appears to offer the most definite and certain means of increasing the productiveness of selfed lines of corn without interfering with their behavior in hybrid combination.

The quantitative relations of the factors under different assumptions are considered, and the bearing of experiments with convergent improvement upon certain theoretical principles is discussed. Such experiments would be of special interest in connection with studies of hybrid vigor, where the principles involved should provide a basis for critical experiments in differentiating between the action of dominant favorable factors and possible physiologic stimulation due to unlike germplasm within the cells.

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REVERSAL OF DOMINANCE AND PRODUCTION OF A SECONDARY SEXUAL CHARACTER IN THE MEDITERRANEAN FLOUR-MOTH

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IN a previous paper[1] the writer has presented data indicating that in the Mediterranean flour-moth, *Ephestia kühniella* Zeller, black (wings and body), b, acts as a simple Mendelian recessive to normal gray, and sooty (base and margin of wings), S, acts as an irregular dominant grading in some cases into normal. Sooty appears to bring about a lightening of color in the mid-area of the primaries at the same time that base and margin are darkened. Homozygous sooty, SS, is more effective than heterozygous, Ss.

Normal "gray" moths vary in depth of color from very light to very dark and in quality of color from silver to tan or from steel-gray to brown. There appear to be two kinds of pigment which may vary in amount and in relative proportion. Minor variations in markings are likewise to be noted. These variations are hereditary, for it has been possible to select lines of uniform type differing markedly from each other. On account of the grading nature of these differences it has not been possible to analyze their heredity although 2412 moths—1162 males and 1250 females—in cultures not involving black have been bred in addition to the data presented in the previous paper. Certain minor hereditary variations, notably "dark base" and "dark mid-area" previously discussed, may cause confusion in distinguishing sooty from non-sooty.

Black females by homozygous gray males have given 684 gray—334 males and 350 females, while the recipro-

[1] Whiting, P. W. "Genetic Studies on the Mediterranean Flour-moth, *Ephestia kühniella* Zeller." *Jour. Exp. Zool.*, Vol. 28, No. 3, July, 1919.

cal has given 703 gray—323 males and 380 females. From similar crosses there were likewise 289 gray of undetermined sex.

Heterozygous males and females have given 1026 gray —533 males and 493 females, and 336 black—169 males and 167 females.

Heterozygous females by black males have given 1276 gray—589 males and 687 females, and 1288 black—616 males and 672 females.

Black females by heterozygous males have given 963 gray—452 males and 511 females, and 898 black—460 males and 438 females.

Black by black have given 1309 black—656 males and 653 females.

If the above fraternities involving black be summarized with the totals of 1919 we have

BB × bb = 2775 gray
Bb × Bb = 4379 gray, 1305 black
Bb × bb = 2733 gray, 2615 black
bb × bb = 1423 black

In the black moths, due to density of pigmentation, sooty is not readily distinguishable unless it occurs in the homozygous condition—in other words, black tends to reverse the dominance of sooty. It has been suggested that this reversal be indicated by reversing the symbols for the factors; thus gray heterozygous for sooty is BBSs while black heterozygous for sooty is bbsS. The latter appears more or less uniformly black while the homozygote, black sooty, bbSS, has a perceptibly lightened mid-area.

With reference to the two loci, Ss and Bb, there are to be expected nine kinds of individuals giving 81 possible types of matings. If sex be disregarded there are 45. In 24 of these one parent at least is BB and hence all offspring are gray. Of the remaining 21 three do not involve sooty, 18 are shown in Table I with ratios of offspring theoretically expected when black reverses the

TABLE 1

THE EIGHTEEN POSSIBLE CROSSES INVOLVING SOOTY AND PRODUCING BLACK; THEORETICAL RATIOS EXPECTED IF BLACK REVERSES THE DOMINANCE OF SOOTY

Designation	Type of mating	Offspring			
		Sooty	Type	Black Sooty	Black
A1	BbSS x BbSS	3		1	
A2	BbSS x bbSS	1		1	
a	bbSS x bbSS			1	
B1	BbSs x BbSs	9	3	1	3
B2	BbSs x bbsS	3	1	1	3
b	bbsS x bbsS			1	3
C1	BbSS x BbSs	6		1	1
C2	BbSS x bbsS	2		1	1
C3	BbSs x bbSS	2		1	1
c	bbSS x bbsS			1	1
D1	BbSS x Bbss	3			1
D2	BbSS x bbss	1			1
D3	Bbss x bbSS	1			1
d	bbSS x bbss				1
E1	BbSs x Bbss	3	3		2
E2	BbSs x bbss	1	1		2
E3	Bbss x bbsS	1	1		2
e	bbsS x bbss				1

dominance of sooty. Ratios containing both gray and black moths, some of which are heterozygous for sooty, are unusual, for these heterozygotes are sooty if gray, non-sooty if black. Eight new ratios are thus shown—B1, B2, C1, C2 and C3, D1, D2 and D3, E1, E2 and E3.

Table II presents experimental data with types of matings arranged and designated as in Table I. Data already published are set in italics. Fraternities or summaries of similar fraternities are numbered for reference. Totals may be compared with numbers expected on the basis of ratios given in Table I. Departures from these expectations are to be noted.

Distinction between gray and black is always clear. Among the gray, sooty and type may be distinguished, for "dark base" and "dark mid-area" are probably ab-

TABLE 2

SEGREGATIONS SHOWING REVERSAL OF DOMINANCE OF SOOTY BY BLACK AND DEPARTURES FROM THEORETICAL EXPECTATION DUE TO SEX AND TO MULTIPLE DARKENING FACTORS.

Designation	Number	Type of mating	Offspring: Sooty ♂	Sooty ♀	Sooty Total	Type ♂	Type ♀	Type Total	Black sooty ♂	Black sooty ♀	Black sooty Total	Black sooty or black ♂	Black sooty or black ♀	Black sooty or black Total	Black ♂	Black ♀	Black Total
A1	1	BbSS ♀ × BbSS ♂	131	131					50	18			14		5	5	
	2	*BbSS* ♀ × *BbSS* ♂			*56*						*21*						
		Total			318						89			14			10
		Expected			323 +						108 –						
A2	3	BbSS ♀ × bbSS ♂	131	160					150	46			70		4	34	
	4	BbSS ♂ × bbSS ♀	144	163					140	67			84			1	
	5	*BbSS ?* × *bbSS ?*			*46*						*50*						
		Total			644						453			154			39
		Expected			645						645						
a	6	bbSS ♀ × bbSS ♂							93	51	144	16	52	68		18	18
		Expected									230						
B1	7	BbSs ♀ × BbSs ♂	117	111		41	23		15	8					27	36	
	8	*BbSs* ♀ × *BbSs* ♂	*96*	*122*		*33*	*45*		*10*	*7*					*24*	*33*	
	9	*BbSs* ♀ × *BbSs* ♂	*530*	*534*		*208*	*190*		*59*	*44*					*140*	*167*	*10*
	10	*BbSs* ♀ × *BbSs* ♂			*29*			*12*			*1*						
		Total			1539			552			144						437
		Expected			1503			501			167						501
B2	11	BbSs ♀ × bbsS ♂	20	16		6	3		5	5					18	28	
	12	BbSs ♂ × bbsS ♀	63	82		32	20		25	17					63	64	
	13	*BbSs* ♀ × *bbsS* ♂			*6*			*7*			*3*						*4*
		Total			187			68			55						177
		Expected			183 –			61 –			61 –						183 –
b	14	bbsS ♀ × bbsS ♂							43	20	63	21	18	39	108	88	196
		Expected									75 –						224 –

TABLE 2—(*Continued*)

Designation	Number	Type of mating	Offspring: Sooty ♂	Sooty ♀	Sooty Total	Type ♂	Type ♀	Type Total	Black sooty ♂	Black sooty ♀	Black sooty Total	Black sooty or black ♂	Black sooty or black ♀	Black sooty or black Total	Black ♂	Black ♀	Black Total
C2	15	BbSS ♀ × bbsS ♂	112	138					59	41					59	90	
	16	BbSS ♂ × bbsS ♀	54	67					31	23					47	38	
	17	*BbSS* ♂ × *bbsS* ♀			*32*						*16*						*20*
		Total			529						170						254
		Expected			477 −						238 +						238 +
C3	18	BbSs ♂ × bbSS ♀	24	16					11	10					11	17	
	19	*BbSs?* × *bbSS?*			*66*						*42*						*16*
		Total			106						63						44
		Expected			107 −						53 +						53 +
c	20	bbSS ♀ × bbsS ♂							10	5					11	17	
	21	bbSS ♂ × bbsS ♀							63	43					41	61	
		Total									121						130
		Expected									125 +						125 +
D2	22	BbSS ♀ × bbss ♂	36	32	68										40	35	75
		Expected			71 +												71 +
D3	23	Bbss ♀ × bbSS ♂	40	49	89										31	46	77
		Expected			83												83
d	24	bbSS ♂ × bbss ♀													93	108	
	25	*bbSS* ♂ × *bbss* ♀															*58*
		Total															259
		Expected															259
E1	26	*BbSs* ♂ × *Bbss* ♀			*26*			*33*									*30*
		Expected			33 +			33 +									22 +
E2	27	BbSs ♀ × bbss ♂	36	35		32	39								74	78	
	28	BbSs ♂ × bbss ♀	30	42		34	31								70	62	
		Total			143			136									284
		Expected			141 −			141 −									281 +
E3	29	Bbss ♀ × bbsS ♂	19	20	39	13	16	29							37	37	74
		Expected			35 +			35 +									71

sent. Among the black, however, sooty and non-sooty intergraded in certain fraternities, especially those extracted from dark gray, and it was therefore found convenient to group grading cases separately.

In fraternities totaled under A1, total sooty agree closely with expectation while black sooty drop below and the unexpected classes, black and black grading to black sooty, appear. Among the sooty, sexes are equal, while among the black, sooty males are much in excess. This latter discrepancy is partly compensated by the fact that excess of females appears in the intermediate group. The black sooty parents of the new data were extracted from dark gray and this darkening character combined with bb and femaleness counteracted the lightening effect of sooty in the mid-area. A comparable condition is to be noted in the new data under A2 and a. In those fraternities in which sex is recorded under A1, A2, and a, there are black sooty—433 males and 182 females; intermediate—16 males and 220 females; black—9 males and 58 females, totaling 458 males and 460 females, closely comparable to the gray—446 males and 454 females. Departure from expectation is largely due to sex and a secondary sexual difference is brought about by factors for general darkening of the wings combined with sooty and black.

Under B1, B2 and b there are with sex recorded, non-gray moths as follows:

	Black sooty		Intermediate		Black	
	♂	♀	♂	♀	♂	♀
Actual	157	101	21	18	380	416
Expected	139+	134−	0	0	418+	401+

Among the 558 males black sooty are above expectation while black are below. This indicates that some bbsS males have been recorded as black sooty. Among the 535 females black sooty are below expectation while black are above. This indicates that some bbSS females have been recorded as black.

No cultures were made of combination C1. Under C2, C3 and c there are with sex recorded, non-gray moths as follows:

	Black sooty		Black	
	♂	♀	♂	♀
Actual	174	122	169	223
Expected	171+	172+	171+	172+

Among the 343 males there is no significant departure from expectation. Among the 345 females black sooty are below expectation while black are above.

Data of Table II are in general agreement with hypothesis of Table I except that in cultures extracted from dark gray "black" females tend to be much darker than "black" males and ratios are therefore distorted. The character sooty grades into non-sooty in several combinations of "black" even in cultures in which it is clearly separable in "gray."

MUTATIONS: THEIR NATURE AND EVOLUTIONARY SIGNIFICANCE[1]

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ALTHOUGH all intelligent people now accept evolution as an historical fact, recognizing that the present living organisms are descended in unbroken series from previous ancestors through hundreds of millions of years, yet there has never been greater difference of opinion concerning the causes which have brought about this diversification of forms, nor concerning the manner in which phylogenies and relationships should be traced. These two problems of causes and relationships in evolution are closely interrelated, for, we shall see, emphasis on any evolutionary factor as of prime importance will have its effect upon our ancestral grouping of many organisms. We have reached a point where it is possible to interpret mere similarities and differences in diverse ways in their bearings on relationships. This of course has always been possible, ever since evolution was accepted; but the diversity of interpretation is greater now than ever before.

In another respect we have advanced far beyond the previous generation, for we have learned a vast amount concerning the structure of the gametes of organisms and the way in which differences arise in the germplasm and are transmitted by inheritance. Visible gametic differences—in other words, differences in the number, size and structure of the chromosomes—afford in many cases a most valuable additional method of gauging the relationships between a group of nearly related species and varieties. There is now no doubt that, particularly in plants (although the method is by no means without

[1] From a lecture delivered to the Newcastle Literary and Philosophical Society.

significance in animals), the comparison of the chromosomes will become of increasing importance in adjudicating upon the relationships between forms within the narrower cycles of affinity, *i.e.*, the species within a genus, the genera in a family or even the families in an order. The use of this method will also react upon our conception of phylogenies and the way in which some new species arise.

This leads me to remark that the problem of specific diversity is certainly not a problem with a single solution, although many biologists still occupy themselves with the attempt to explain all specific differences and all phylogenies on the basis of one evolutionary factor. That this favored factor is sometimes natural selection, sometimes mutation or hybridization or the Lamarckian factor or orthogenesis in some form (*e.g.*, under the name rectigradations or nomogenesis), simply increases the confusion. For each advocate is intent upon explaining not only the likely but also the unlikely cases on the basis of his favorite evolutionary factor.

The paleontological history of organisms is more closely involved with problems of phylogeny than is the study of living species. Nevertheless the problems of phylogeny and species differentiation must find the same general solution with living as with fossil forms. The difficulty is that on the one hand the evidence of variation, ontogeny and distribution must necessarily be relatively meager in fossils, however well preserved; while on the other hand, with living species the geological time element is lacking.

I am, however, only proposing to discuss mutations in some of their bearings on evolutionary problems. But it is necessary as well to glance at the evidence in certain other directions. As regards the Lamarckian factor, I have advocated elsewhere[2] the view that many characters of animals and plants, particularly those

[2] Mutations and Evolution. Wheldon & Wesley, London, 1921.

showing recapitulation, could be most easily understood if the inheritance of acquired characters had taken place. We are forced, however, to recognize that there is at present no satisfactory experimental evidence that the inheritance of acquired characters occurs. Notwithstanding other negative results one was inclined to accept the experiments of Guyer and Smith on the inheritance of eye defects in rabbits, produced by treatment of the mother rabbit with a cytolysin; but since the rumored appearance of similar eye defects in untreated rabbits of the same stock we have had no further communication on this subject. There is thus at present no substantial experimental evidence on which a claim for proof of the Lamarckian factor can be based. Certain other recent experiments which have been given a Lamarckian interpretation will be referred to later.

The term mutation has of course been used in different senses. Waagen in 1869 applied it to the series-transformations which he found in certain Jurassic ammonites occupying successive horizons, and which he believed to arise from internal causes. These would correspond in some respects with the rectigradations of Osborn. In modern usage, De Vries implied by the term mutation the sudden origin of a new elementary species or variety in one or in a few individuals from the parent species. He emphasized the discontinuity of origin in contrast to Darwin's conception of continuity in connection with natural selection. The work of Morgan and his school on the mutations of Drosophila has shown the Mendelian (*i.e.*, discontinuous) inheritance of many small differences, *e.g.*, in eye color, which if placed together would form a continuous series, but which are nevertheless discontinuous in their origin from the parent form.

As regards the Oenothera mutations, various writers have attempted to make out a plausible case for the view that *Oe. Lamarckiana,* the species in which they were first described, originated as a garden hybrid.

While this view remains purely hypothetical, and contrary to the probabilities, the history of this species is no longer of special significance because similar mutations have been described in a number of wild species of Oenothera in North America. Natural crossing is not, however, eliminated as a possible cause of some of the mutational behavior in the Oenotheras. But the effort to show that the mutations are merely the simple and direct result of crossing has long since broken down.

In Drosophila, the hypothesis of crossing set up by Jeffrey as an explanation of the many mutations occurring has not a single fact to sustain it; for it is well known that the species are sterile with each other, only one pair of very closely related species being known to cross, and these produce entirely sterile hybrids. Innumerable mutations have now been described from a considerable number of wild American and European species of this genus. Everything indicates that the conditions have little or nothing to do with their production, and forms are known to occur in the wild corresponding closely to some of those appearing in captivity.

The charting of over 400 factor mutations in the four chromosomes of *Drosophila melanogaster* is now well known. By crossing experiments with *D. simulans,* the only species with which it can be crossed, Sturtevant and Plunkett[3] have shown that 13 sex-linked mutations in the latter species occupy the same relative positions as in *D. melanogaster.* In the third chromosome, however, a study of seven corresponding (parallel) mutations in the two species indicates that one end of this chromosome has been inverted in one of the species. This is one of various indications that germinal changes may occur from species to species in the arrangement of the material (genes) within the chromosomes.

In Oenothera, specific rearrangements of another kind take place in the chromatin. Thus in several species recently investigated by Miss Sheffield in my laboratory

[3] *Biol. Bull.* 50: 56. 1926.

all the chromosomes form a ring in diakinesis, while in certain other species a pair of chromosomes is always separate from the other 12 which form a ring. In one form, *Oe. rubricalyx,* four pairs of chromosomes are constantly found separate from the remaining ring of six. Thus the passage from certain species of Oenothera to others must be marked by a change in the arrangement of the chromosomes.

In the Composite genus Crepis, M. Navashin[4] has shown another interesting and significant type of difference to exist in the nuclei. For instance, in *C. Dioscoridis,* which has four pairs of chromosomes all distinguishable by their form, one pair (D) has constantly a tiny satellite attached to it by a thread at one end. But in some plants of this species the two satellites are both of equally large size, in some one satellite is large and one small, while in others both satellites are small. Moreover, these three types of plants occur in the ratio 1:2:1 (43:90:42), as would be expected with free assortment of the chromosomes in crossing.

In another species in which two chromosome pairs have an attached satellite, Navashin has shown that two races only exist, those plants in which only one member of this pair has a satellite being about twice as numerous as those in which only one chromosome has a satellite. By the examination of young embryos it was shown that individuals in which both chromosomes lack the satellite are incapable of development. In other words, the absence of the satellite from both chromosomes of a pair has a lethal effect. As representing another kind of structural change, Taylor has recently found one type of the common onion in which one of the chromosomes has, instead of a single satellite, two arranged tandem.

Such examples as these—and there are many others—show that germinal changes of many kinds occur in the chromosomes, and that the phylogeny of chromosomes plays an important part in the phylogeny of species.

[4] *Zeitsch. f. Zellforsch.* 4: 197. 1926.

Turning now to another aspect, let us consider briefly the origin of geographical subspecies or varieties in their relation to mutations. Do they represent separate evolutionary processes, or are they fundamentally the same? Osborn has recently expressed the view that geographical subspecies arise gradually and continuously with intergrades from the species. He proposes[5] to apply to this process the term speciation, which has hitherto been used in a general sense as meaning differentiation of species by any method. The word has been used in this general sense for many years[6] and it seems desirable that, if it is to be used at all in future, this general sense of the term should be adhered to. Osborn, using the term in his new sense, concludes (*l.c.*, p. 40) that "speciation is a normal continuous process; it governs the greater part of the origin of species; it is apparently always adaptive. Mutation is an abnormal and irregular mode of origin which while not infrequently occurring in nature is not essentially an adaptive process; it is, rather, a disturbance of the regular course of speciation."

In support of his point of view, Osborn quotes the results of Sumner, who has shown by breeding experiments with various geographic subspecies of the mouse, *Peromyscus maniculatus,* from different parts of California that these subspecies remain stable under changed environmental conditions, and that forms from different areas when bred under the same environment do not converge towards a common type. The fact that these geographical differences are inherited is important, but it tells us nothing as to how these differences arose. On the basis of his experiments, for example with pale-colored desert forms, Sumner was inclined to believe that geographic subspecies arise gradually as a non-adaptive response to the environment, and from crosses

[5] Osborn, H. F., 1927. The origin of species V: speciation and mutation, AMER. NAT. 61: 5–42.

[6] See, for example, Gates, The mutation theory and the species-concept, AMER. NATURALIST 51: 579. 1917.

he was inclined to conclude that the inheritance of the differences was non-Mendelian. Were this true generally of geographic subspecies, there would be some basis for distinguishing, as Osborn does, between them and mutations as regards their origin and evolutionary significance. Such contrasts were formerly pointed out by me,[7] with special reference to North American owls as well as other animals and plants.

But Sumner has recently[8] found another situation, from a study of a different species, *Peromyscus polionotus,* in Alabama and Florida. As the facts are of much interest they may be briefly outlined. On the Island of Santa Rosa, a narrow bank of very white sand about 50 miles long stretching along the Gulf of Mexico close to the coast, is found a form, *leucocephalus,* which is almost white, and which has been called a distinct species, solely on account of its geographical isolation. It is evidently a geographic subspecies of *P. polionotus.* Farther inland, on dark soil, is found the widely distributed subspecies *polionotus,* while an intermediate subspecies *albifrons* is found along the coast.

Now Sumner states that, contrary to his earlier conclusions, these subspecific color differences show striking Mendelian segregation, the parental coat patterns being recovered in quite small F_2 populations. This must mean that quite a limited number of Mendelian factor differences are involved. If such is the case, any fundamental difference between the origin of new species by mutation or by the formation of local subspecies has yet to be shown. It appears probable that these two processes are much more nearly allied than has been realized, and they may prove with further analysis to be essentially identical. Sumner agrees that the "white" mouse

[7] The mutation theory and the species concept, AMER. NAT. 51: 577–595. 1917.

[8] Sumner, F. B., 1926. An analysis of geographic variation in mice of the *Peromyscus polionotus* group from Florida and Alabama, *Journ. Mammalogy* 7: 149–184, pls. 4, figs. 9.

of Santa Rosa Island is adapted for living in the white sand, although he adheres to the view that the pale colors of the desert varieties are non-adaptive in character. In this subspecies *leucocephalus* we appear to have an adaptive form which has arisen under isolation through a few Mendelian mutations. We can only allude here to the numerous cases described by Chapman and by Stresemann in birds, in which mutations have apparently given rise to new species and subspecies.

One other aspect of mutations can be briefly touched upon. In the last 20 years many investigators have announced the experimental production of mutations in plants or animals, but unfortunately in many cases, through lack of adequate controls, there was no certainty that the aberrant forms which appeared were the result of the treatment. In the more recent experiments of Dr. Heslop Harrison[9] this connection between the treatment and the appearance of new forms seems clear. In these important results, melanic variations of *Tephrosia bistortata* and other Lepidoptera have appeared in small numbers in the offspring derived from larvae which had been fed on smoke-contaminated foliage or on certain salts. The later inheritance in crosses is of the ordinary Mendelian type.

From an analysis of these experiments it seems clear that the melanism appears as the result of a germinal change in certain of the germ nuclei of a few of the treated individuals, this germinal change being either produced or at any rate rendered more frequent by the treatment. Clearly we have to do here with a case of induced mutations; and it is unfortunate that the experiments have been given a Lamarckian setting, for they really have no direct bearing on the question of the inheritance of acquired characters.

In conclusion we are compelled to recognize that mutations, *i.e.*, discrete changes in the germ plasm, are of many kinds, some of them "spontaneous," some related

[9] See Harrison and Garrett, Proc. Roy. Soc., Vol. 99B. 1926.

to crossing, some environmentally induced. They must have played an important rôle in the production of species and varieties, both in plants and animals. The increasing recognition of parallel mutations and convergences, both in plants and animals, has an effect of fundamental importance in the tracing of phylogenies. For parallel development must mean the independent occurrence of the same series of variations in unrelated forms. Modern phylogenies tend to deal less with divergences and more with parallelisms or convergences. This is shown, for instance, in the recent conclusions of Bower on the phylogeny of the ferns. It would appear, however, that some of the phylogenetic conclusions of Berg (*Nomogenesis*) go much farther than the facts justify. But the recognition that divergences, convergences and parallelisms are all of frequent occurrence adds greatly to the complexity of phylogenetic study.

THE MIGRATION OF ANIMALS FROM THE OCEAN INTO FRESHWATER AND LAND HABITATS

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VARIOUS lines of evidence indicate that animals originated in the ocean and have since Cambrian times gradually invaded land and freshwater habitats (6, pp. 15–19). Apparently no great groups arose in the past except in the ocean (9). The blood and body fluids of many animals resemble sea water, which is an adequate medium for supplying the materials for the building of living substance. This paper considers briefly the probable routes and causes of migrations from the ocean.

An animal going from the ocean into freshwater usually encounters more variable temperatures and of course enters a less saline medium. The body fluids become more dilute or adjustments occur which prevent loss of salts from the body of the animal and keep its blood at a density near that which it had in the ocean. Certain crabs tolerate considerable variations in the salt content of their blood, which contains salts in about the same quantities as the surrounding medium. Hence they readily migrate into brackish or nearly fresh water. Marine bony fishes, on the other hand, have blood which contains about half as much salt as sea water. Some of these fishes can swim into freshwater without harm (4). Their blood becomes somewhat diluted, but after a time tends to return to the original condition which it had in the ocean (1, 17).

In general, marine animals die in freshwater on account of loss of essential body constituents. They vary in their ability to control interchange with the surrounding medium. They have two lines of defense:—the membranes

on the outside of the body and the membranes on the outside of the cells. A freshwater animal is never in exact osmotic equilibrium with its environment. When changes in the latter occur, however, a new internal balance is attained. Freshwater animals have in gaining ability to retain salts to some degree lost power to adjust their internal fluids to chemical variations in the surrounding medium. The integument of mammals permits little or no interchange, that of amphibians exerts some selective action, and that of annelids offers practically no resistance to the passage of salt solutions (1). Some marine species could perhaps become established in freshwater if their larval stages were as resistant as adults to variations in the salt content of surrounding media.

Animals that live in the ocean enjoy relative thermal stability compared with those in freshwater or land habitats. Some animals in beach pools along the Canadian Coast are confined to lower strata because they can not endure an increase of 3° C. in temperature (11). Many fishes which make migrations into freshwater are very sensitive to changes in temperature and are quick to choose variations which tend toward their optima (4). Animals which live in freshwater or on land ordinarily are able to survive considerable variations in temperature.

When an animal leaves fresh or salt water to take up life in the atmosphere, it must:—(1) endure extreme variations in temperature, (2) attain the ability to acquire oxygen from the atmosphere, and (3) develop means to continually acquire or to prevent the loss of water. Animals which remain in the comparatively stable ocean are usually able to remain active throughout the year, but those which live in habitats where temperature and water vary often hibernate or estivate during certain seasons.

The respiratory exchange of a turtle, which is a representative poikilothermic animal, varies roughly in proportion to the temperature (7). This means that such an animal lives more slowly at low temperatures, and below certain limits must become behavioristically inactive.

During hibernation a turtle requires little oxygen, but it is not wholly inactive physiologically. Homoiothermic animals are largely independent of environmental temperature variations. A penguin remains active throughout the year and is able to persist in the antarctic cold where a frog or a turtle would be unable to live. Some land mammals, though maintaining a comparatively high and constant temperature during certain seasons, are forced to hibernate during others. The Columbian ground squirrel, though it probably takes no food during hibernation, is not wholly inactive for its sexual glands enlarge greatly in preparation for reproduction during the next season (18). The activities of land insects are greatly influenced by temperature and some are strikingly adjusted to seasonal changes. Some hibernate and probably insects generally show seasonal variations in blood concentration (12).

The respiration of a land animal is not very different from that of an aquatic animal. Gases are exchanged through a wet membrane in either case. Water contains comparatively little dissolved oxygen, and animals that live in it commonly breathe through vascular integuments or branching gills. Land animals live in a medium which contains a higher percentage of oxygen. They must acquire oxygen through wet membranes and these are usually enclosed in internal cavities in order to protect them from desiccation. The walls of such cavities are often stiffened by chitin, cartilage, or bone to prevent collapse.

Desiccation is a serious matter for land animals. Most types are well protected by an impervious integument, which may be supplemented by setae, scales, feathers, hairs, or other structures. The land amphibians are necessarily somewhat restricted in distribution on account of their moist coverings. They are commonly found in moist, shady situations, or are nocturnal. When a locality on land is subject to extreme temperatures and is also arid, animals must be highly adapted. In this con-

nection, two rodents belonging to genus Cynomys in Turkestan are of interest (10). One is active for only 3½ to 4 months during the year, when desert vegetation is available. It estivates during the dry fall season and continues to be inactive as a hibernator during the winter. The other rodent lives where there is more vegetation. It hibernates in winter, but does not estivate.

Routes

In the consideration of the migrations of animals from the ocean, there have been ardent advocates of this route or that. Such views are not altogether justifiable, for there has probably been no definite limitations to particular avenues. Species of animals tend to extend their ranges continually and since the Cambrian Period have with each opportunity moved into favorable freshwater and land habitats as they became available. There have been thousands of routes in all parts of the earth. The five following are only groupings which indicate the general nature of aboceanic movements.

Subterranean Route. Thomson (20) believes that worms spread from the aquatic habitats into the soil. The facts that the relatives of soil worms are found largely in freshwater and that species of marine worms do not live in freshwater make it appear that the soil was probably populated from types which had become adjusted to freshwater. The skins of earthworms are semipermeable and do not readily permit the loss of salts from the body fluids (1). The ability of the burrowing marine worms to endure variations in salinity and the optimum salinities for their activities are not known and should be studied.

River Route. There are at present a number of species of fishes and crustaceans which are found both in the ocean and in rivers. Some move at irregular intervals back and forth from river to ocean; others make periodic migrations. The minnow, *Fundulus heteroclitus* (Linn.), lives readily in the ocean or in freshwater and commonly swims

in and out of the mouths of rivers. At least thirty-three species of fishes which readily adjust themselves to ocean or freshwater are known (4). A number of species of prawns have similar abilities. The river crabs in Northern South America show varying degrees of adjustment to life on land. They are primarily associated with rivers but some species spend most of their time hiding under logs and similar shelters during the day and come out at night to forage. They do not occur along the seashore (14). A number of species of fishes, like the salmon and shad, migrate into streams to lay their eggs and certain eels move from rivers into the ocean to spawn. There is abundant evidence that rivers have long served as highways for the migrations of animals. Movements from the ocean into streams appear to be most easily accomplished in the tropics or, in temperate regions, during seasons when temperatures in rivers are not very different from those in the ocean.

Adjustment of Animals to Freshwater through the Gradual Dilution of the Ocean. In various parts of the earth bodies of water have been separated from the ocean and gradually diluted until in some cases they became quite fresh. There are especially good examples of such transformations in Scandinavia, where crustaceans (Mysis, Pontoporeia, Chiridothea, etc.) which differ but little from those in the ocean are found in inland lakes. In the deep lakes in the interior of northern North America, similar crustaceans and representatives of the herring family are found. Such animals can endure wide ranges of variation in salinity but are usually quite susceptible to high temperatures. They probably became marooned in the depths of inland lakes by migrating in from the ocean at the close of the last continental glaciation. Pelseneer (16) believes that in some parts of the earth (Indo-China) the ocean continually is so diluted by heavy rainfall that animals more readily become adjusted to freshwater.

Marsh and Swamp Route. The amount of water in marshes and swamps commonly varies greatly at different seasons. Many animals in such habitats are quite resistant to desiccation and often endure prolonged periods of drought. A number of swamp fishes can remain without water for weeks or months. Barrell (3) maintained that bony fishes originated in freshwater, that recurring aridity caused certain of them to become adjusted to air breathing, and thus led to the origin of land vertebrates. On account of their shallowness and stagnation, swamps are often quite deficient in oxygen. The snails are commonly pulmonates and some species are even amphibious and spend considerable time out of water. Many of the insects are air breathers.

Route through Intertidal Zone. Without a preliminary baptism in freshwater, many animals have undoubtedly left the ocean and attained directly a life on land. Along ocean beaches are snails, crustaceans, and fishes which have various degrees of ability to live on land (6). The little beach amphipod, Orchestia, and the crab, Ocypoda, drown in freshwater and the ancestors of these crustaceans probably never had any residence in it. Fiddler crabs (Uca) live in the intertidal zone. They are active only when the tide is out, but carry sea water in their branchial chambers. The ghost crab (Ocypoda) moistens its gill chambers at intervals with sea water but does not carry water. Certain of the land crustaceans, such as Birgus and Geacarcinus, have rudimentary gills and breathe through pulmonary cavities. The land hermit-crab, Cenobita, breathes through a vascular abdomen which is safely enclosed within a snail shell. These land crustaceans return to the ocean once each year to leave their young for a short stay in their ancestral marine home.

All migrations have not been away from the ocean. Some types of animals have, after becoming adjusted to life on land or in freshwater, gone back to the ocean. The insects, which appear to have originated on land, have

sent midges and bugs to represent them in the ocean. The sea snakes and sea turtles spend their lives at sea, but return each year to the land to breed. Perhaps the salmon and shad originated in freshwater and came later to spend a part of each generation at sea. Certain frogs, lizards, and mites commonly hunt along the seashore. Mites are inundated at high tide, tadpoles develop in brackish water, and some lizards swim out a little way into the ocean and dive for sea-weed. The albatrosses and petrels rarely see land, except when they breed. The seals also come ashore to breed, but the whales have left the land forever.

CAUSES OF MIGRATION

There has been, and is, some doubt as to the probable causes which have led animals to leave the ocean and freshwater for land, but the writer believes that the five following have reasonable support by scientific evidence.

Breeding. The snails of the genus Littorina which live on the ocean beaches show an interesting series of differences in reproduction which are adapted to differences in habitats (6). Three species live in the intertidal zone:—*L. littorea* is found near low tide mark, lays large numbers of eggs which hatch as early veligers, and the larval life is long; *L. obtusata* lives in the middle of the intertidal zone, lays a smaller number of eggs which hatch as fully formed veliger larvae, and the free-swimming larval life is very brief; *L. neritoides* lives near the high tide mark and is viviparous. These three species of snails show the characteristic suppression of free-swimming larval stages, which is often associated with the migration of animals from the ocean. In the swamps in Colombia there lives a snail which crawls out of the water and deposits its clusters of beautiful, rose-colored eggs on the sticks above the water.

Among fishes there are a number of species (salmon, shad, etc.) which, when mature, leave the ocean and enter freshwater to spawn. Still more remarkable is the case

of the grunion, a little, sardine-like fish, which along the California coast comes inshore in spring when the highest tides which occur at the time of full moon are beginning to ebb. It wiggles out on sandy beaches above the water and buries its eggs beneath the sand. Two weeks later the young hatch when they are washed out by the spring tides which come with the dark phase of the moon (19).

Food. There is usually an abundance of food in the drift which is deposited by falling tides and in the plants and animals which live in the intertidal zone. Many animals came to forage at low tide. Among the crustaceans the crabs, beach fleas, and some of the isopods came from the ocean. The beach snails had the same origin. Even the fishes are represented by the beach-skipping gobies which hunt in the intertidal zone. The land has also sent representatives:—birds, rats, lizards, beetles, flies, spiders, mites and even frogs. The jetsam from the sea furnishes food and shelter for an abundant population.

Safety. One who has watched fiddler crabs on exposed beaches when the tide comes in has no doubt that these crustaceans are afraid of the ocean. As the tide flows near the entrance of a fiddler burrow, the owner digs a pellet of mud with which he securely barricades himself within until the tide recedes again (13). The ghost crabs (Ocypoda) show every sign of fear when pursued. They throw sand over their bodies and protrude watchful eyes. They never enter the ocean to feed but forage over the beaches above it (5). The gobies which live on tropical beaches are very reluctant to enter the water and when in danger skip about on land or over the top of the water. There appears to be no reason why the grunion should with great effort wriggle out of the ocean to bury its eggs, except to provide safety for its offspring.

Aridity. Barrell (3) says, "the numerous examples of fishes, mostly tropical, which can remain active out of water are almost all fishes of freshwater origin. The rarity of the passage of crustaceans, gastropods, and ver-

tebrates from a truly marine to a truly terrestrial mode of life through the apparently open path of the tidal zone adds to the evidence that an unused food supply could not alone operate as a cause sufficient to induce this change, for so far as this factor is concerned the river faunas would have no clear advantage over those in the tidal zone." Barrell believes that fishes arose in freshwater, that some types were forced to evolve into land animals by aridity, and that they were little influenced by the "lure of oxygen" or food. He says of pulmonary respiration that, "it is an adaptation which has been forced repeatedly to a greater or less degree on fishes by the recurrence of an unfavorable environment rather than one assumed within a constant environment because of inherent advantages." The behavior of lung fishes, certain galaxids, and other swamp fishes during dry seasons gives support to Barrell's views (8).

Oxygen. Probably lack of oxygen has rarely, if ever, been a factor in causing animals to leave the ocean for land, but it appears to be of some importance in swamps and marshes. Dr. L. E. Noland has recently made observations, as yet unpublished, which show that shallow plant-filled water may have its oxygen completely used up during the night by organic decay and the activities of organisms. The fact that such situations are commonly inhabited by pulmonate and often amphibious snails, tracheate arthropods, and air-breathing fishes makes one wonder if respiration has not been a factor in causing animals to take up land life. The tracheate arthropods appear to have originated on land and those which have returned to water have in most cases not given up air breathing. The land snails and fishes came from aquatic ancestors. Perhaps need for safety and available food caused them to migrate to the land, but lack of oxygen may also have been a factor. More information is needed in this connection in regard to the gaseous content of the water in such situations as mangrove and cypress swamps, and other situations where shallow water which varies in depth stagnates over muddy bottoms.

Conclusions

The ecological and physiological basis on which the migration of animals from the ocean to freshwater and land and from freshwater to land depends offers many points of theoretical and practical interest and should be better understood than it is at present. The body fluids, regulation of temperature, toleration of temperature fluctuations, respiration, relations to lack of water, reproduction, food and enemies of the animals concerned should be more thoroughly known.

There is good evidence that certain animals have in the past attained to land life through the intertidal zone, and that others have migrated into rivers or swamps and from them gained the land. There is also evidence that marine animals have continued to live in water which became gradually fresh. It is possible that burrowing animals along the seashore have gradually moved inland in the subterranean stratum.

The causes which appear to have led animals to leave the ocean are opportunities for breeding, food and safety. Those which have been concerned with the movement of animals from freshwater to land appear to have been aridity and perhaps also lack of oxygen.

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SHORTER ARTICLES AND DISCUSSION

THE EPISTATIC EFFECT OF VESTIGIAL IN DROSOPHILA

THE problem of determining the extent of the epistatic effect of vestigial wing character in Drosophila crosses was developed from results obtained in the cross *vestigial* × *taxi*. This initial experiment was planned to learn if inheritance of size of wing in two such recessive forms was governed by multiple factors. As the problem progressed other matings were made of vestigial with: jaunty, clip, and miniature, all recessives, and each being longer than vestigial.

Vestigial × *Taxi.*

In the cross vestigial × taxi careful measurements, under binoculars, were made of wing lengths of 2,005 flies. These included the parental types (wild, taxi, and vestigial), F_1, F_2, 536 double recessive flies, and 413 true vestigials derived from the F_2 vestigial flies. The mean wing lengths and variability coefficients of these types are shown in Table I.

TABLE I

Parents	*Long Wings*						
	♀ M, 2.929	C_v	7.89	± 0.357	n,	111	
	♂ 2.628		6.06	± 0.286		102	
	Taxi						
	♀ M, 2.787	C_v	4.67	± 0.238	n,	87	
	♂ 2.507		7.62	± 0.330		121	
	Vestigial						
	♀ M, 1.136	C_v	11.15	± 0.069	n,	58	
	♂ 1.182		12.97	± 0.0812		58	
F_1	*Long Wings*						
	♀ M, 3.39	C_v	2.437	± 0.140	n,	69	
	♂ 3.04		2.538	± 0.146		69	
F_2	*Long Wings*						
	♀ M, 3.39	C_v	4.98	± 0.232	n,	104	207
	♂ 3.00		3.82	± 0.180		103	
	Taxi						
	♀ M, 3.09	C_v	6.14	± 0.535	n,	30	58
	♂ 2.715		6.25	± 0.562		28	
	Vestigial						
	♀ M, 1.134	C_v	16.06	± 0.974	n,	62	116
	♂ 1.162		18.42	± 1.196		54	

Double Recessive Cultures

♀ M, 1.137	C_v	13.41	± 0.374	n, 293
♂ 1.193		18.11	± 0.553	243

True Vestigials derived from F_2

♀ M, 1.1497	C_v	14.60	± 0.532	n, 210
♂ 1.236		14.73	± 0.553	203

After examining the variability coefficients of these types, especially that of the vestigial flies, it seemed probable that wing length could be increased by selection among the vestigials. To effect this numerous cultures were made of extra long winged flies, both double recessives and true vestigials, but with negative results as far as carried out.

In the wild and taxi types the wings of the females are longer than of the males, but among the vestigials this condition is reversed. This was due to a larger number of males having longer wings, which caused the increased variability.

As will be seen from the distribution in F_2, the phenotypes have segregated out in approximately a 9 long: 3 taxi, 4 vestigial ratio. Therefore the double recessive type is phenotypically vestigial, and among the vestigials there are three genotypes: (a) homozygous vestigial, vvTT, (b) heterozygous vestigial, vvTt, and (c) double recessive, vvtt. Identification of the three genotypes was made by mating them to taxi, whereupon they behaved as follows:

(a) vvTT × VVtt (taxi) ..... all long wings.
(b) vvTt × " ..... ½ long, ½ taxi,
(c) vvtt × " ..... all taxi.

In view of these facts it is considered that a single factor difference is responsible for the two types of flies. The item of chief interest disclosed by this cross, however, was the demonstrated epistatic effect of vestigial over taxi, a wing mutation decidedly longer than vestigial. It then remained to determine to what extent vestigial is epistatic to other wing mutants.

Other Vestigial Crosses.

Vestigial flies were then crossed with jaunty, clip and miniature flies, all characters differing from the wild wing type, but each longer than vestigial. In these crosses all the flies of F_1 were of the wild type, and in Table II are shown only the F_2 distributions.

TABLE II

A. *Jaunty × Vestigial*

	long		jaunty		vestigial		Total
Observed	248		68		95		411
Calculated	231		77		103		411
Deviations	17	± 6.78	9 ± 5.19		8	± 5.92	

B. *Clip × Vestigial*

	long		clip		vestigial		Total
Observed	159		63		76		298
Calculated	167.5		56		74.5		298
Deviations	8.5	± 5.78	7 ± 4.35		1.5	± 5.04	

C. *Vestigial × Miniature*

	long		miniature		vestigial		Total
Observed	141		42		57		240
Calculated	135		45		60		240
Deviations	6	± 5.18	3 ± 3.95		3	± 4.52	

(Original Cross)

D. *Vestigial × Taxi*

	long		taxi		vestigial		Total
Observed	207		58		116		381
Calculated	215		72		94		381
Deviations	8	± 6.53	14 ± 4.95		18	± 5.70	

In each case the double recessive class has been vestigial, clearly exhibiting the epistatic effect of vestigial over all characters concerned. From the analysis of the cross *vestigial × taxi* it developed that the wing length of the double recessive vestigial was no longer than that of the stock vestigials, or the F_2 vestigials, and the inference seems warranted that a similar situation exists in the last three crosses.

Discussion.

The double recessive genotypes derived from each of these four crosses would furnish excellent laboratory material for class use, since all would be phenotypically vestigial, but each would give peculiar breeding results when crossed with the wild and hypostatic flies as compared with the true vestigial stock. Crosses of the double recessives with the hypostatic stocks would give all hypostatic flies, whereas crosses of the true vestigials with the hypostatics would give all wild type flies. Crosses of the double recessives with wild stock, and F_1 backcrossed to the double recessive parent, would disclose three phenotypes, instead of the two obtained with the true vestigial stock, one of them being the hitherto unknown hypostatic stock.

It is hoped that in the near future study will be made of the behavior of vestigial when crossed with a dominant wing mutant, like Dichaete.

All four crosses demonstrate the epistatic effect of vestigial over the wing length mutants considered, all of which are longer than vestigial, and indicate that: (1) this effect is probably more widespread than now suspected, and (2) the distribution of classes in F_2 points to single factor differences, rather than multiple factors.

UNIVERSITY OF CALIFORNIA CHARLES F. POOLE

A DOMINANT BODY COLOR IN DROSOPHILA REPLETA

AMONG a large number of Drosophila repleta collected at the Zoological Laboratory of the University of Pennsylvania in September, 1923, there was found a female with very light body color in contrast to the dark body color of the wild type. The ground color of the body was yellow instead of gray; the blackish-brown spots at the bases of the bristles were entirely lacking or light brown in color. The dark abdominal bands and other markings which are usually dark were also light brown. The body color of the fly was very similar to that of the mutant "yellow" in Drosophila melanogaster; however, the bristles were little if any different from those of the wild type.

This female was mated with wild type males and gave the following offspring: yellow males, 95; yellow females, 96; wild type males, 104; and wild type females, 92.

The offspring of this cross were mated with the following results:

Wild type when mated together gave all wild type offspring.

Wild type when mated with yellow gave wild type and yellow in equal numbers.

Yellow mated with yellow gave three yellows to one wild type.

Offspring from yellow × yellow were mated with these results:

Yellows when mated together gave either three yellows to one wild type or all yellows.

Yellows mated with wild type gave either equal numbers of yellow and wild type or all yellows.

From these data it appears that the factor for yellow is dominant and that flies homozygous for yellow are viable. So far as known to me this is the only dominant mutant body color in any species of Drosophila.

The stock has been lost due to the difficulty of raising the species under laboratory conditions.

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